

An Experimental Study of the Digestive System  
of the Horse.

by

Frank Alexander.

A thesis presented for the degree of  
Doctor of Science in the University of Edinburgh.



Diseases of the digestive tract account for nearly half the deaths which occur in horses; despite this, very few investigations have been made into the functions of this system. The work described here was undertaken with the object of providing some information on this subject. It is hoped that the information obtained will be of value in understanding some of the pathological conditions and will also advance knowledge of digestion in herbivorous mammals. In this last respect, studies on the horse provide a useful comparison with those on the ruminant.

Since the size and cost of horses made acute experiments impracticable, it was necessary to devise other methods of investigation. A supply of fresh intestines was available from horses slaughtered for food. This was utilized and a technique developed for the perfusion of isolated segments of intestine (Alexander, 1949). The movements of the ileum and small colon were studied in this fashion and also the effect on them

of various humoral agents. When the ileum was perfused with isotonic solutions containing oxygenated red blood cells, it showed propulsive movements. This was studied in more detail (Alexander, 1950), and it was found that the magnitude of the movements varied with the supply of oxygen. The effects of the inorganic constituents of the perfusion fluid were investigated and, of these, only sodium ions seemed to have a specific effect. During the course of these experiments a fast propelling mechanism was observed which propelled balloons through the ileum at rates of 1-4 cm./sec. This mechanism could be shown only in a small proportion of preparations and differed from the slow propulsion described in the first paper.

The movements of the horse stomach were investigated (Alexander, 1951a) and it was found that the perfused isolated stomach did not contract strongly enough to expel its contents. Attention then turned to the living animal and a method devised to record gastric contractions by means of a tube passed down the oesophagus. Gastric contractions were unaffected by histamine, carbamylcholine or posterior pituitary extract.

By mixing carbon granules of suitable size with food, it was possible to measure the rate of

passage of food residues through the alimentary tract of the horse (Alexander, 1946). In view of the length and complexity of this system in the horse it was surprising to find that the time taken by digesta to traverse the tract was approximately the same as in man. A more detailed study became possible with the development of radiographic methods (Alexander and Benzie, 1951). So far as could be ascertained, this was the first publication of radiographs of the equine digestive tract. It was of particular interest to discover that digesta remained in the stomach and small intestine of the suckling foal for a longer time than in the weaned animal.

Since abdominal surgery in the horse has been regarded as particularly hazardous, the study of digestion by means of permanent fistulae into the intestine had not been attempted. However, the development of a suitable technique seemed essential and a method was evolved for the fistulation of the caecum (Alexander and Donald, 1949). The success of this procedure encouraged the extension of the intestinal surgery to include fistulation and exteriorisation of the small intestine (Alexander, 1951b) and now it has been found possible to fistulate the caecum, ventral and dorsal colons in the same animal (Alexander, 1952a).



The ileal fistulae allowed the repetition on the living animal of some of the experiments on the perfused ileum. There appeared to be an association between ileal motility and blood flow (Alexander, 1952b) which supported the observations on the in vitro preparations. The fistulae of the large intestine were used to study movements, products of fermentation and cellulose digestion. Three types of movement were recognised but no evidence of antiperistalsis was obtained. Large amounts of the lower fatty acids were found in the caecum and large colon and the proportion of the various acids differed between the dorsal and ventral colon. It appeared that withholding food depressed cellulose digestion (Alexander, 1952a).

The experiments on the small intestine had been confined to a study of the mechanical factors. However, by utilizing the fistulated animals it was possible to study absorption. It was found that feeding glucose produced a hyperglycaemia which was associated with the presence of a high glucose concentration in the ileum. Since no glucose or its fermentation products appeared in the caecum, it seemed probable that it was completely absorbed in the small intestine. This may explain, in part, the finding that the concentration of glucose in horse blood is about 50% higher than in ruminants (Alexander, 1954a).

Since the horse is a good example of a herbivore in which microbial digestion is carried out in the large intestine, studies of digestion in the horse are complementary to those on the ruminant, animals in which microbial digestion takes place in the stomach. The digestive processes in these species have been reviewed (Alexander, 1954b) and it is suggested that the horse is particularly suited for studies of cellulose digestion.

During the development of the surgical techniques described, considerable experience was gained in anaesthetising ponies for long periods. A variety of anaesthetics were tried and eventually a satisfactory technique evolved. This has been described in detail and an account given of the anaesthetic deaths which occurred during the course of the operations (Alexander, 1954c).

The development of the techniques described has encouraged investigation of the microbial activities of the horse's digestive tract. So far, a specific streptococcus, a lactate fermenting organism (*Veillonella gazogenes*) (Alexander, Macpherson and Oxford, 1952) and a glucuronide decomposing enzyme have been isolated from the horse colon. This enzyme resembled others which occurred at sites of cellulose in various herbivores (Marsh, Alexander and Levvy, 1952)

It has been suggested that the enzyme is concerned with the digestion of hemicellulose.

The Rate of Passage of Food Substances through the Digestive Tract of the Horse.

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Part 2

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# THE RATE OF PASSAGE OF FOOD RESIDUES THROUGH THE DIGESTIVE TRACT OF THE HORSE

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## INTRODUCTION

In view of the complexity of the equine digestive tract considerable interest has been aroused in the time taken for food residues to traverse it. This phenomenon has been studied in man and the common laboratory animals by a variety of methods, but, excepting the observation of Fish (1923), no similar studies appear to have been carried out on the horse. The methods so far employed have depended on the addition of small coloured beads to the food (Elliot and Barclay-Smith, 1904), the admixture of an inert insoluble colouring matter (Guernsey and Evvard, 1913), or roentgenological studies

TABLE I  
THE FAECAL EXCRETION OF INGESTED CARBON GRANULES

Horse	Observation No.	Time of appearance of indicator in faeces	
		Beginning	End
Chestnut mare ...	1	18.5 hours	44.5 hours
	2	19.0 "	
	3	25.0 "	58.0 "
	4	22.0 "	44.0 "
	5	23.0 "	39.0 "
	Mean	21.5 "	46.4 "
Bay mare ...	1	18.5 hours	68.5 hours
	2	18.0 "	50.0 "
	3	44.0 "	67.0 "
	4	22.5 "	40.0 "
	5	18.0 "	40.0 "
	6	22.0 "	44.0 "
	Mean	23.8 "	51.6 "
Bay mare (2) ...	1	21.0 hours	48.0 hours
Black mare ...	1	23.0 hours	47.0 hours
	2	23.0 "	55.0 "
	3	24.0 "	46.0 "
	4	21.0 "	39.0 "
	5	21.5 "	39.5 "
	Mean	22.5 "	45.3 "
Chestnut gelding ...	1	22.0 hours	37.0 hours
Mean of all observations ...	...	22.6 $\pm$ 1.32 (S.E.)	47.4 $\pm$ 2.26 (S.E.)

of the rate of passage through the gut of a barium meal. In the experiments described here carbon particles were mixed with the food and the rate of their excretion in the faeces observed.

#### METHODS

The method employed in these experiments was to keep the animals on a maintenance diet of oats 6 lb., bran 2 lb., and hay 12 lb. One hundred gm. of granular carbon were mixed with a normal feed of oats and bran and the time of feeding noted. All faeces passed after this feed were carefully examined for the presence of carbon. The granules used were of about the size of a mustard seed and could easily be seen with the naked eye. The examination was facilitated by shaking the faeces with water in a cylinder when much of the fibrous matter floated off.

Carmine, ferric oxide, kaolin and beads were tried as markers but proved unsatisfactory. The first three substances were difficult to detect, whilst the last was not recovered even by the end of one week.

#### RESULTS

The results of these experiments are tabulated in Table 1.

#### DISCUSSION

The paucity of data about the progress of food residues through the equine digestive tract prompted further investigation, using granules of carbon as the indicator. This substance fulfilled the criteria of Alvarez (1940) in that it had little bulk compared to the total amount of the meal, it mixed intimately with the usual food, and was easy to detect. Furthermore, in view of the observations of Hoelzel (1930) that substances with a high specific gravity tend to lag behind the food residue, it was important to choose a material having a density similar to the normal food constituents. It was found that granular carbon met this requirement. Roentgenological studies were, of course, impracticable.

The results of these experiments show that the time taken for the food residue of a normal meal to traverse the equine digestive tract is approximately the same as Burnett (1923) and Alvarez (1940) have found for man. In view of the great disparity between the human and equine digestive tracts in both their relative lengths and complexities this observation is striking and the explanation not obvious.

#### SUMMARY

Carbon granules mixed in a feed of oats and bran and fed to horses appeared in the faeces in 22.6 ( $\pm 1.32$ ) hours and continued up to 47.4 ( $\pm 2.26$ ) hours after feeding.

The figures were the means of seventeen separate observations.



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THE ACTION OF SOME HUMORAL AGENTS ON THE HORSE  
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of Physiology, Royal (Dick) Veterinary College, Edinburgh.

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THE ACTION OF SOME HUMORAL AGENTS ON THE HORSE  
INTESTINE. By FRANK ALEXANDER. From the Department  
of Physiology, Royal (Dick) Veterinary College, Edinburgh.

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INTRODUCTION.

ALTHOUGH the alimentary tract of the horse presents many interesting anatomical features, and despite the fact that disturbances of this system account for a large proportion of disorders in the adult animal, few investigations into the mechanics of the intestinal tract have been carried out. Some observations on intestinal movement were made by Colin [1886], who found that waves, described as antiperistaltic, were common in the small intestine of the horse. Unfortunately, his observations were conducted on the intestine of the freshly killed horse without any precautions to prevent drying and cooling. More recently Tanaka and Ohkuho [1940], using the method of Magnus, investigated the action of adrenaline on the ileum of the horse and found that it caused a contraction. Since the observations of Colin and the experiments of Tanaka and Ohkuho gave somewhat unexpected results, it seemed important to extend their studies. An investigation was therefore undertaken into the effects of adrenaline, acetyl choline, histamine and posterior pituitary extract on the activity of the horse's intestine.

METHODS.

Pieces of gut, about 20 cm. in length, were taken from a horse immediately after destruction and put in cold Tyrode's solution. Strips of about  $1 \times 10$  cm. were cut from the larger pieces and suspended in Tyrode's solution in a bath of 100 c.c. capacity. The strips were taken from the following regions: ileum, cæcum, large colon, small colon and rectum. The strips of ileum were cut in a longitudinal direction; great difficulty was experienced in getting responses from strips cut in a circular fashion despite the use of the technique recommended by Siaulis and Sollmann [1927]. The anatomical arrangement of the muscle-fibres in the large intestine facilitated the separation of the longitudinal and circular fibres, the former being gathered into tæniæ [Sisson, 1921]. Strips of tænia and circular fibres were studied separately. The muscle layers of the rectum were easily dissected free from each other, and from mucous membrane, into circular and longitudinal bundles.

Solutions of the drugs were prepared by diluting a 1/1000 solution of adrenaline in Tyrode's solution and by making fresh solutions of acetyl choline and histamine acid phosphate. A preparation of posterior pituitary (Burroughs Wellcome) containing 10 units per c.c. was used.

Before any tests were made each strip was suspended in the bath for at least 30 minutes. The drug under investigation was added to the bath in increasing concentration until a measurable response was obtained. The dose required to produce this effect is shown in the appropriate figure. When an effective concentration had been reached, a dose ten times as big was added to make sure that the response first obtained was not a specific response due to the presence of the drug in high dilution. Gruber [1922] found that adrenaline in high dilution caused contraction of the frog's intestine whereas stronger solutions gave the opposite effect.

#### *Perfusion Technique.*

In order to study the effect of these drugs on intestinal propulsion, other experiments were devised in which the gut was maintained in a viable state by the perfusion of its blood-vessels with physiological saline solutions or blood.

A length (about 1 m.) of ileum or small colon, supplied by one intestinal artery, was removed from a freshly killed horse. The artery and vein were cannulated and the open ends of the intestine tied over glass tubes of suitable diameter. The loop of bowel was then laid on a sheet of perspex and placed in a heated box the temperature of which was thermostatically controlled at 36–38° C. All the blood was washed out of the organ by perfusing with Tyrode's solution at 37° C. In the early experiments the perfusion pressure was controlled by raising or lowering a bottle fitted with a Mariotte tube; later this was replaced by a pump. The perfusion apparatus is illustrated by fig. 1.

Tyrode's solution, blood, washed red cells and plasma were used as perfusion fluids. When defibrinated blood was employed, some difficulty was experienced from the presence of vasotonins and, while it was possible to remove them by first passing the blood through a separate intestinal loop, it was preferable to overcome this obstacle by using chlorazol fast pink as anti-coagulant.

Experiments were conducted in which the effect of different perfusion fluids on the propulsion of boluses, seeds and fluids were observed. The action of drugs on intestinal movements was measured by recording the response on a smoked drum using a volume recorder connected to the glass tube in the end of the segment.

To perfuse the cat's bowel, a cat was etherised and bled by severing the carotid arteries; ligatures were placed round the oesophagus and



ascending colon, and the aorta rapidly dissected from its attachments to the roof of the abdomen. This allowed the liver, stomach and intestines to be removed from the body by cutting above the œsophageal ligature and below the colic. These organs were placed in a dish of Tyrode's solution while the remaining operations were completed.

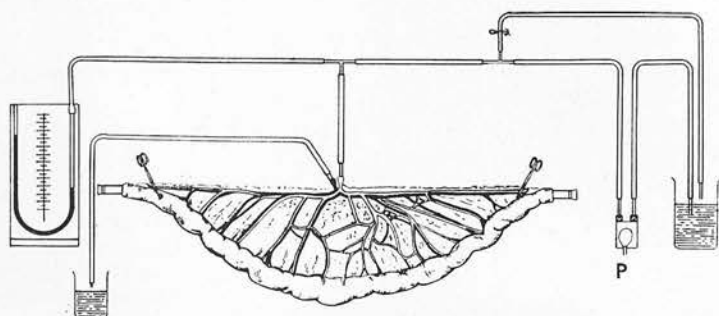


FIG. 1.—The arrangement for perfusing the isolated horse intestine.  
P: pump and valves.

Cannulæ were introduced into the cranial mesenteric artery and into the common trunk of the portal vein. After placing a ligature round the anastomosis between the duodenal branch of the cœliac and first intestinal arteries, the liver and stomach were removed and the intestines placed in a heated box and perfused in a manner similar to that used for horse intestine.

## RESULTS.

### *Intestinal Strips.*

The results of the experiments on intestinal strips are summarised in Table I. The most remarkable of these was the marked increase in tone produced by adrenaline on the ileum (fig. 2) and circular fibres of the large intestine (fig. 3, A). This effect was reversed by ergotoxine figs. 2 and 3, A).

Two cases, in a series of fifty experiments on the horse ileum, occurred in which adrenaline caused inhibition of rhythmic contractions without any increase in tone. Strips of the circular muscle of large and small colon were not so consistent in their response to adrenaline; inhibition of rhythmic contractions occurred in about 25 per cent. of preparations from the large colon, and there was no response to adrenaline in about 40 per cent. of preparations from the small colon.

Strips cut from the region of the pyloric and ileocæcal sphincters were suspended in the bath and their response to drugs tested. In the few instances in which a response was obtained, the effect of adrenaline was to increase the tone.

TABLE I.—THE EFFECT OF ADRENALINE, ACETYL CHOLINE, HISTAMINE AND POSTERIOR PITUITARY EXTRACT ON INTESTINAL STRIPS FROM THE HORSE.

Drug.	Tissue.	Effect.	
		Tone.	Rhythm.
Adrenaline . . . . .	Ileum	Increased	Unchanged
	Cæcum:		
	Circular strips	Increased	Inhibited
	Tænia	Unchanged	Inhibited
	Large colon:		
	Circular strips	Increased	Increased
	Tænia	Unchanged	Inhibited
	Small colon:		
	Circular strips	Increased	Increased
	Tænia	Unchanged	Inhibited
	Rectum:		
	Circular strips	Increased	Increased
Acetylcholine . . . . .	Longitudinal strips	Unchanged	Inhibited
Histamine . . . . .	All	Increased	Increased
Posterior pituitary extract	All	Unchanged	Unchanged

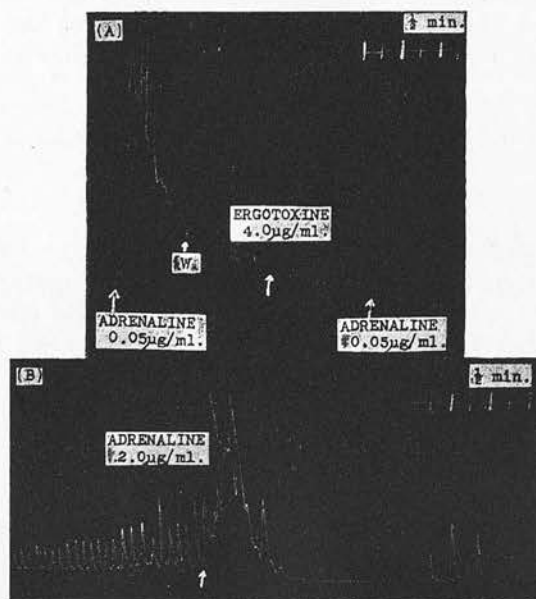


FIG. 2.—A. Usual effect of adrenaline on a strip of ileum.  
 B. Diphasic action of adrenaline on the ileum seen in a few cases.

It was noted that the rate of rhythmic contraction in strips of tænia from the small colon was about three times as fast as in strips of tænia



from the large colon or cæcum. Further, the dose of adrenaline required to inhibit rhythmic contractions of cæcal and large colon

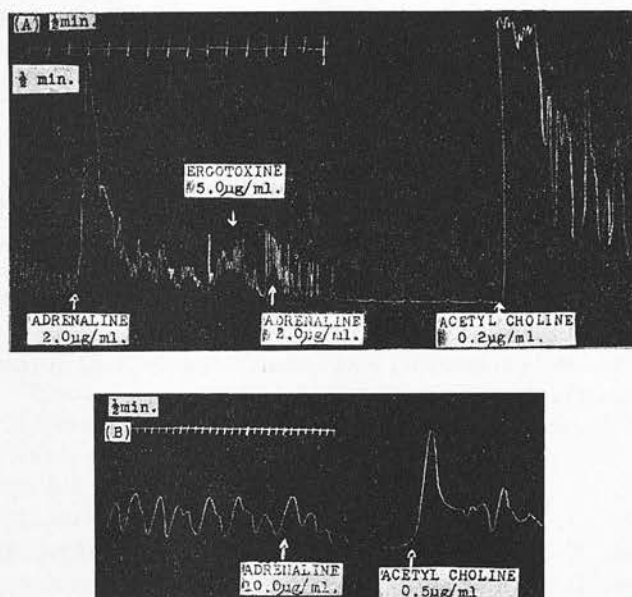


FIG. 3.—A. The action of adrenaline and acetyl choline on a circular strip of large colon.

B. The action of adrenaline and acetyl choline on a strip of large colon tænia.

tæniæ was greater than needed to inhibit those of the small colon tæniæ (fig. 3, B).

The responses to acetyl choline (fig. 3) and histamine (fig. 4) were consistent, and easily elicited from all preparations.



FIG. 4.—The action of histamine on (A) ileum, (B) large colon tænia, (C) small colon tænia, (D) rectum.

*Perfusion Experiments.*

When loops of horse ileum were perfused with oxygenated Tyrode's solution, slow, inco-ordinated, pendular movements and feeble waves of contraction, running orally and aborally, were observed. These movements did not cause any progression of objects placed in the lumen of the bowel.

Perfusion of the bowel with defibrinated blood induced strong movements of the following types: peristalsis, antiperistalsis, pendular movements, and a spiral twisting of the gut on its mesenteric border. The predominant type of movement was a progressive segmentation; no peristaltic rushes were seen. These movements produced an interesting variety of effects on different objects placed in the lumen. Boluses of cotton-wool and soft paraffin, about 3 cm. in diameter, were moved to and fro; the overall progression was towards the oral end of the segment. The following experiment illustrates this phenomenon:—

*Expt. 38.*

Perfusion fluid—defibrinated blood.

Perfusion pressure—70 mm. Hg.

14.15. Bolus placed in the middle of the loop of bowel.

14.30. Bolus had progressed 10 cm. towards oral end.

14.45. Bolus had progressed 15 cm. towards oral end.

Seeds and inert particles were propelled both orally and aborally, but more particles were propelled towards the aboral end of the bowel than the reverse. This is shown graphically in fig. 5.

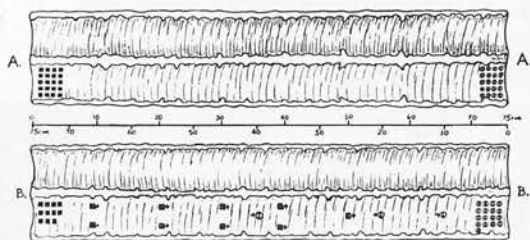


FIG. 5.—A-A shows the seeds placed at each end of a loop of ileum. B-B shows their position 2 hours later, the intestine having been perfused with defibrinated blood for this period. The oral end of the loop is on the left.

In other experiments a glass tube, closed by rubber tubing and a spring clip, was introduced through the bung (fig. 1) at each end of the segment. The lumen of the ileum was filled with normal saline solution. When the two ends were simultaneously unclipped and the outflows measured, it was found that saline was expelled at each end of the segment. The following experiment illustrates this finding:—

	Oral outflow.	Aboral outflow.
16.35	Perfusion begun	
16.40	180 c.c.	30 c.c.
16.45	10 c.c.	15 c.c.
16.50	10 c.c.	55 c.c.
16.55	0 c.c.	55 c.c.
17.00	5 c.c.	10 c.c.

*Investigation of the Perfusion Fluid.*

To determine the fraction of blood responsible for these propulsive movements, red cells were separated by centrifugation, washed in Tyrode's solution and restored to their original volume with Tyrode's solution. When this solution was used for the perfusion of the ileum, the same movements were observed as with defibrinated blood as the perfusing fluid; similar propulsion took place. Perfusion with plasma produced an increase in movements which rarely persisted for more than 30 minutes, and was much weaker than that produced by perfusion with washed red cells. The movements produced by perfusion with blood or washed red cells were maintained for at least 3 hours.

Graphic records of the variations in intraluminal pressure produced by perfusion with Tyrode's solution, plasma and washed red cells are shown in fig. 6.

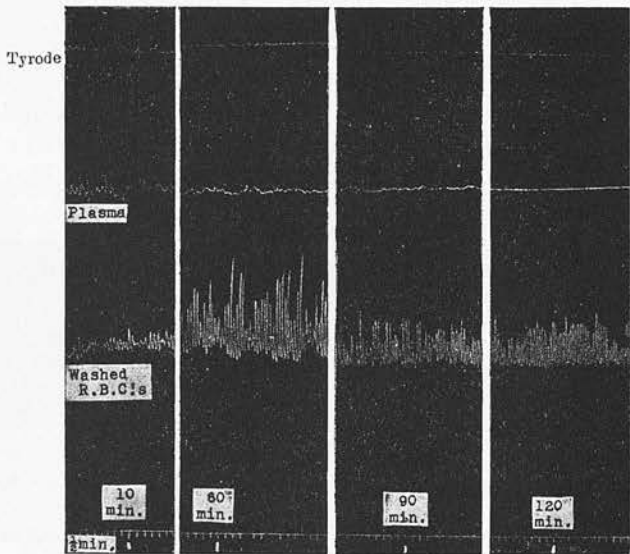


FIG. 6.—The effect of different perfusion fluids on the movements of the perfused ileum. The time from the start of perfusion is shown in minutes.

*Perfusion of the Ileum of the Cat.*

In this species propulsion occurred with Tyrode's solution as the perfusing fluid. Propulsion was always in an aboral direction. The following experiment was typical:—

Ileum of Cat Perfused with Tyrode's Solution through  
the Cranial Mesenteric Artery.

11.45.	Pea introduced into duodenum.
12.05.	Pea had progressed 5 cm. aborally.
12.10.	„ „ „ 9 cm. „
12.25.	„ „ „ 20 cm. „
12.40.	„ „ „ 20 cm. „

*Perfusion of the Small Colon.*

When this organ was perfused with oxygenated Tyrode's solution weak movements were observed. The predominant type was a rippling movement of the haustra, which seemed to have the effect of kneading the contents. Slow peristaltic and antiperistaltic waves and an occasional contraction of the tæniæ were seen.

Perfusion with blood or a suspension of washed red cells produced the same types of movement but increased in strength. The increase in strength was not so great as in the ileum. These movements did not cause any progression of the normal contents of the small colon or of artificial boluses introduced into the lumen.

*The Effect of Drugs on the Perfused Bowel.*

The various drugs were injected into the arterial cannula of the perfused bowel. The total volume of the perfusing fluid was about one litre. The results of these experiments are given in Table II. The

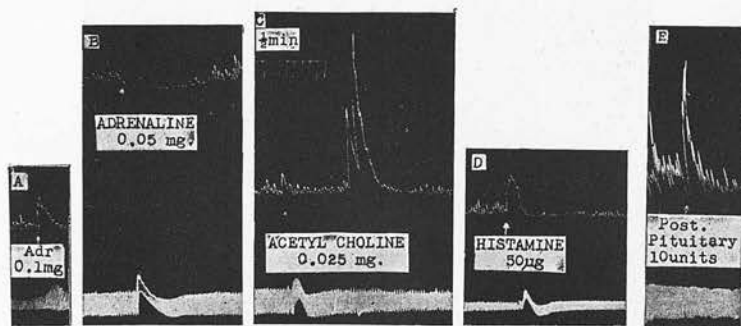


FIG. 7.—(A), the effect of adrenaline on the Tyrode-perfused ileum; (B), (C), (D), (E), the effect of various drugs on the blood-perfused ileum.

effects of drugs on the perfused ileum are shown in fig. 7, and on the perfused small colon in fig. 8.

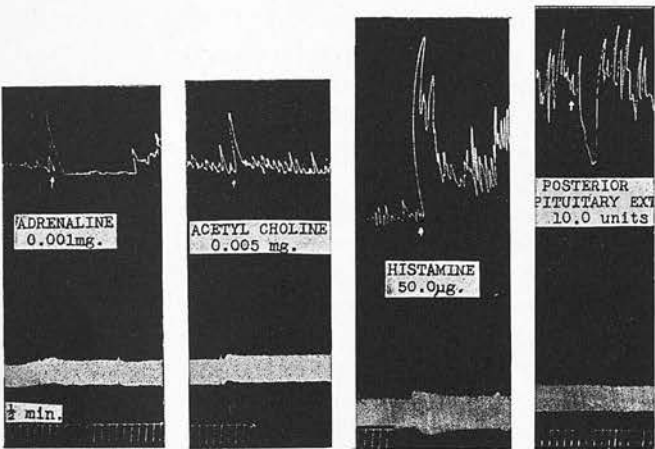


FIG. 8.—The effect of various drugs on the blood-perfused small colon.

TABLE II.—THE EFFECT OF DRUGS ON THE PERFUSED BOWEL OF THE HORSE.

Drug.	Organ.	Perfusion fluid.	Effect.	
			Tone.	Rhythm.
Adrenaline . . .	Ileum	Tyrode's	Increased	Unchanged
	Ileum	Blood	Decreased	Inhibited
	Small colon	Blood	Increased	Inhibited
Acetyl choline . . .	Ileum	Blood	Increased	Increased
	Small colon	Blood	Increased	Increased
Histamine . . .	Ileum	Blood	Increased	Increased
	Small colon	Blood	Increased	Increased
Posterior pituitary extract .	Ileum	Blood	Increased	Increased
	Small colon	Blood	Increased	Unchanged
	colon	Blood	Decreased	Inhibited

DISCUSSION.

*Action of Drugs on Intestinal Strips.*

The experiments described have confirmed the findings of Tanaka and Ohkuho [1940], and, further, have shown that the excitor effect of

adrenaline on the horse's intestine was not confined to the ileum. This effect of adrenaline was demonstrated on circular strips of cæcum, colon and rectum. It was independent of the tone of the tissue or the concentration of adrenaline. It was important to show the effect independent of these conditions, since Brown and McSwiney [1927] found the action of adrenaline on strips of muscle from the stomach of rabbits varied with their tone. Similarly, Gruber [1922] found dilute solutions of adrenaline caused contraction of the isolated frog intestine whereas concentrated solutions produced inhibition.

It was possible, by adding ergotoxine to the bath, to change the excitor effect of adrenaline on these tissues of the horse into one of inhibition. Though adrenaline has a diphasic effect on the activity of several organs, it has not so far been shown to affect the intestine in this way. A satisfactory explanation of the diphasic action is that first proposed by Dale [1906], who suggested that these viscera may have both excitor and inhibitor adrenergic fibres in their nerve-supply. Acetyl choline produced characteristic effects on the intestinal strips. Histamine, however, had the interesting property of inducing strong rhythmic contractions, an effect which was particularly marked on strips of tænia. Posterior pituitary extract showed no activity when tested by this method. Since this substance finds some employment clinically for the stimulation of intestinal movement, it is important to test its action on the intestine by other methods.

#### *Perfusion Experiments.*

The main purpose of the intestinal movements is to mix the ingesta with the intestinal secretions and, having exposed the mixture to the mucosa, to propel the residue aborally. To study these movements satisfactorily it is necessary to devise a preparation in which a length of intestine can be observed as a whole. With this in view, the method of perfusing segments of bowel was developed, and it was hoped this preparation would facilitate the investigation of the factors influencing propulsion.

The perfusion experiments on the ileum showed that unless red blood cells were present in the perfusing fluid propulsion did not occur, and that adequate oxygenation was an important factor in the co-ordination of the bowel movements. The possibility of this feature being peculiar to the horse was at once considered, and similar experiments were performed on the ileum of the cat. These experiments showed that perfusion with oxygenated Tyrode's solution enabled the ileum of the cat to propel objects placed in the lumen.

It is clear from these experiments that differences exist between the factors controlling intestinal propulsion in the different species. This



finding is supported by the work of van Liere and his co-workers [1943], who found that anoxia decreased intestinal propulsion in mice but had no effect in dogs. Similarly, Carnot and Glenard [1912] and Glenard [1913] observed peristaltic movements in the Locke perfused intestine of the dog and studied the progress of objects through the lumen.

It seemed possible, from these findings, that the dietetic habits of the species might determine the degree to which anoxia interfered with intestinal propulsion, herbivores being more sensitive to this condition than carnivores. Moreover, Alvarez [1937] found that anoxæmia completely disrupted the peristaltic rush in rabbits. This finding, however, was not in agreement with the results obtained by von Oettingen, Sollmann and Ishikawa [1928]. These latter authors found that long segments of rabbit ileum, suspended in oxygenated Locke solution, propelled various liquids along the lumen by means of peristaltic waves. They stated, however, that an intraluminal pressure of 15 mm. water was necessary to elicit propulsion; hence the ensuing peristalsis might well have been due to the simple distension of the gut, as in Trendelenburg's preparation. It would have been interesting if von Oettingen and his colleagues had investigated the effect of increasing the intraluminal pressure to similar levels at the caudal end of the segment.

#### *Analysis of Movements of Perfused Ileum.*

The movements of the perfused ileum were difficult to analyse. The predominant type was a progressive segmentation which frequently merged into a slow wave of peristalsis. These movements were shown to propel the intestinal contents both orally and aborally, a somewhat unusual observation which is in agreement, however, with the early work of Colin [1886], who, observing the bowel movements in recently killed horses, noted the occurrence of rings of constriction, running both orally and aborally, which pushed the contents of the small intestine from duodenum to ileum and ileum to duodenum.

Since the experiments on the ileum of the cat, performed with an identical perfusion technique, did not show any antiperistaltic propulsion, it seemed unlikely that the antiperistalsis recorded on the ileum of the horse was an artifact. A possible function of this movement might be the more complete mixing of the intestinal contents, and it was noted that mixtures of gum acacia and carbon were soon evenly spread through the perfused loop. Similarly, London [1911] found that no matter the size of meal consumed, it was evenly distributed over the ileum so that all parts shared in the work of absorption. In the experiments on the perfused ileum of the horse, the trend of propulsion was in an anal direction except when large boluses were present. In this case the



purpose of the antiperistalsis might have been to reduce them to a smaller state of division, since normally the ileum never contains large particles.

### *Movements of the Small Colon.*

The movements of the perfused small colon were confined, almost exclusively, to the haustra. A rolling movement of the haustra was the most common, although both peristaltic and antiperistaltic waves were observed. Contraction of the antimesenteric tænia was seen occasionally.

In view of the results obtained on the ileum it was surprising that perfusion with neither Tyrode's solution nor blood produced any marked degree of propulsion in the small colon, no matter whether the normal contents or artificial boluses were in the lumen. The work of Langley and Magnus [1905], however, indicated that the colon of the rabbit possessed an intrinsic propelling mechanism. They found that segments of the distal colon of the rabbit, suspended in physiological saline solution, pushed faecal pellets from the cranial to the caudal end. Since the distal colon and small colon are analogous structures, it seemed that the failure of the isolated small colon of the horse to propel its contents might be due to (a) separation from the central nervous system, (b) disruption of continuity of the alimentary tract, (c) injury to some sensitive structures caused by the experimental manipulations. These points require further elucidation. It may be noted that Alvarez [1939] made the interesting suggestion that the functions of the stomach and colon are controlled by the central nervous system to a much greater degree than is the small intestine.

### *Effect of Drugs on the Perfused Bowel.*

The experiments on the effect of drugs on the perfused intestine showed similar results to these observed on the Magnus preparation, with the exception of adrenaline. This substance caused contraction of the isolated strip and Tyrode's perfused ileum, whereas it completely inhibited the contractions of the blood-perfused ileum. These results were somewhat difficult to reconcile; a possible explanation might be that, as the contractions of the blood-perfused ileum were propulsive, they were, to some extent, co-ordinated by nervous mechanisms. The effect of adrenaline in stopping them might have been due to its action on such a co-ordinating mechanism. In the case of the isolated strip and Tyrode's perfused ileum the action might have been solely on the muscle, due to the co-ordinating mechanism being in abeyance as a result of anoxia.

Of the substances studied, the only one which increased the activity

of isolated strips, blood-perfused ileum and blood-perfused small colon was histamine. The results of the experiments with posterior pituitary extract offered no support for the use of this drug to stimulate intestinal movements. Indeed, its use seemed to be contra-indicated.

The results of the experiments on the isolated tænia, in which tæniæ from the small colon showed a faster rate of contraction than those from the large colon and cæcum, do not accord with Alvarez' [1939] gradient theory of intestinal activity. A possible explanation of this, however, may lie in the histology of the tænia. Cæcal tæniæ and tæniæ of the large colon consist mainly of fibrous tissue with little smooth muscle, whereas tæniæ from the small colon are composed almost entirely of smooth muscle [Sisson, 1921]. Nevertheless, considering the activity shown by strips of tæniæ, it seems unlikely that their sole function is to support the circular fibres as suggested by Austoni [1937].

#### SUMMARY.

The actions of adrenaline, acetyl choline, histamine and posterior pituitary extract on the intestine of the horse have been investigated. The Magnus preparation and a perfusion technique were used. The perfusion method is described in detail.

Propulsive movements of the horse ileum were only produced when the perfusion fluid contained red blood cells. The cat ileum performed propelling movements when perfused with Tyrode's solution.

In the horse ileum both oral and aboral propulsion occurred, aboral propulsion predominating. The small colon did not propel its contents even when perfused with blood.

Excepting adrenaline, the various humoral agents gave similar effects on the Magnus preparation and on the perfused bowel. Adrenaline produced a rise in tone with little change in the rhythmic contractions in isolated strips of ileum, Tyrode-perfused ileum and isolated strips of the circular muscle of the large intestine; whereas it inhibited the movements of the blood-perfused ileum and small colon. Acetyl choline and histamine caused a rise in tone and usually increased the rate of rhythmic contractions. Posterior pituitary extract produced little effect on the various preparations of intestine.

#### ACKNOWLEDGMENTS.

I wish to thank Professor J. H. Gaddum, F.R.S., for his advice and encouragement, Mr. D. M. Douglas for criticising the manuscript, Professor W. M. Mitchell for laboratory accommodation, and the Agricultural Research Council for a grant to carry out this work.

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# CONTENTS

	PAGE
CASTILLO-NICOLAU, J. DEL, and SCHWEITZER, A. Reflex Rebound in Extensor Muscles . . . . .	1
ALEXANDER, FRANK. The Action of some Humoral Agents on the Horse Intestine . . . . .	11
DUKE, HELEN N. The Action of Carbon Dioxide on Isolated Perfused Dog Lungs . . . . .	25
CULLUMBINE, H., and KOCH, A. C. E. The Changes in Plasma and Tissue Fluid Volume following Exercise . . . . .	39
RAKSHIT, PRABHASCHANDRA. Communicating Blood Vessels between Bronchial and Pulmonary Circulations in the Guinea-Pig and Rat . . . . .	47
LATHE, G. H., and PETERS, R. A. Some Observations on the Comparative Effects of Cold and Burns on Protein Metabolism in Rats . . . . .	55
HALL, KATHLEEN. The Rôle of Progesterone in the Mechanism of Pelvic Relaxation in the Mouse . . . . .	65

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FACTORS INFLUENCING THE MOTILITY OF THE PERFUSED  
HORSE INTESTINE. By FRANK ALEXANDER. From the  
Department of Physiology, Royal (Dick) Veterinary College,  
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# QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY

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FACTORS INFLUENCING THE MOTILITY OF THE PERFUSED HORSE INTESTINE. By FRANK ALEXANDER. From the Department of Physiology, Royal (Dick) Veterinary College, Edinburgh.<sup>1</sup>

*(Received for publication 2nd December 1949.)*

AN investigation has been conducted into the effect on the motility of the perfused horse ileum of anoxæmia, of varying the rate of perfusion and of varying the ionic constituents of the perfusate. In addition, observations on the mode of propulsion along the gut have been made.

METHODS.

The technique for the perfusion of isolated loops of horse ileum has been described earlier [Alexander, 1949]. A record of the venous outflow was obtained by means of a Condon tipper. The various perfusion fluids were prepared by separating the red cells from defibrinated horse blood and restoring the volume with the appropriate isotonic solutions. Intestinal motility was recorded from a small balloon in the lumen of the gut connected to a water manometer. A constant pressure of about 10 cm. water was used and the fluctuations in pressure recorded with a McDowall volume recorder.

In the experiments devised to study the mode of propulsion in the intestine, a small balloon 4–5 cm. in diameter was fixed to small calibre tubing and inserted into the oral end of the intestine. The time taken for the balloon to traverse the length of bowel was noted. In some experiments the perfusion apparatus was arranged so that two or three loops of bowel could be perfused simultaneously from the same reservoir of blood and under identical conditions.

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## RESULTS.

*Effect of Anoxæmia.*

When the ileum of the horse was continuously perfused with a solution of red cells suspended in Tyrode's solution without re-oxygenation, the magnitude of the contractions showed a marked decrease. The rate of contraction was unaffected by this procedure. Oxygenation of this perfusion fluid restored the contractions to their original magnitude (fig. 1, A). This was shown in each of four preparations.

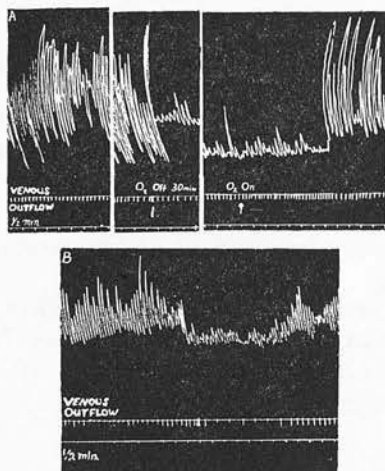


FIG. 1.—A. The effect of oxygen on the motility of the perfused intestine.  
B. The effect on motility of variations in the venous outflow.

*Effect of the Rate of Perfusion.*

It was found, from observations of the venous outflow, that the magnitude of the contractions depended on the amount of blood perfusing the loop of gut in unit time. A fall in the venous outflow caused a decrease in magnitude of the contractions, which was restored by increasing the venous outflow (fig. 1, B).

*Effect of Varying tonic Constituents of the Perfusate.*

The effect of varying the other constituents of the perfusion fluid was next investigated. Unfortunately, these variations were limited to varying the solution in which the red cells were suspended, as it was impossible to supply sufficient oxygen for the horse gut to show measurable movements without using red cells.

(a) *Isotonic Saline Solution.*—Perfusion of the ileum with red cells

suspended in a 0.9 per cent. solution of sodium chloride maintained strong rhythmic contractions for more than three hours. These movements could not be distinguished from those produced by red cells suspended in Tyrode's solution.

(b) *Isotonic Sucrose Solution*.—Experiments were then conducted to determine the extent to which the sodium and chloride ions were essential for the perfused gut to maintain its motility. Since the sodium in horse blood is confined to the plasma [Smith, 1921], it was possible to replace this element entirely by substances such as sucrose.

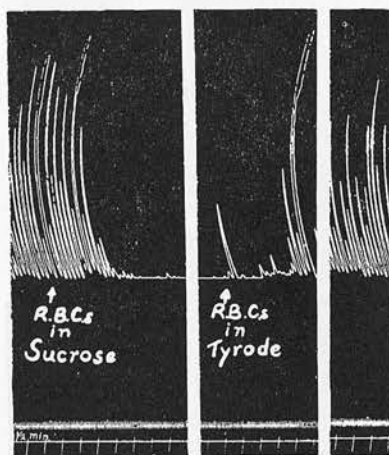


FIG. 2.—The effect of perfusing the ileum with red cells suspended in isotonic sucrose.

When this was done and the gut perfused with red cells suspended in isotonic sucrose solution, the movements of the bowel very quickly stopped (fig. 2). In each of four experiments they were almost completely restored by re-perfusing with red cells suspended in Tyrode's solution.

(c) *Isotonic Glucose Solution*.—The effect of glucose differed from that of sucrose in that it was irreversible. In four experiments perfusion with red cells in isotonic glucose was followed by perfusion with red cells in Tyrode's solution, but in no case did motility return to that obtained before perfusion with glucose.

(d) *Mixture of Isotonic Solutions of Saline and Sucrose*.—When various mixtures of isotonic solutions of sucrose and sodium chloride were used to suspend the red cells and the gut perfused with such mixtures, it was found that, provided the mixture contained more than 20 per cent. of isotonic saline, motility not easily distinguishable from that produced by perfusion with red cells in Tyrode's solution was maintained.

(e) *Other Sodium Salts*.—Further experiments were performed in which different salts of sodium were used for the suspending solution, and it was found that isotonic solutions of sodium bromide, phosphate (pH adjusted to 7.4) and sulphate maintained the motility of the perfused bowel just as well as sodium chloride (fig. 3). An isotonic solution of sodium iodide irreversibly inhibited motility (fig. 4).

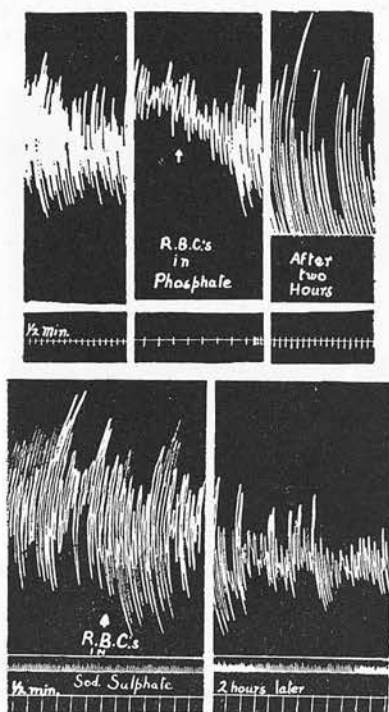


FIG. 3.—The effect of perfusing the ileum with red cells suspended in isotonic solutions of sodium sulphate and sodium phosphate (pH 7.4).

(f) *Other Kations*.—Since red cells were essential for the horse ileum to show measurable motility, it was not possible to investigate the effect of the absence of ions such as potassium and calcium, because both these elements are present in the red cell. When potassium or calcium was added to a mixture of red cells and 0.9 per cent. saline in an amount sufficient to restore the proportion of these elements to that of Tyrode's solution, no change of motility was observed. The addition of excess potassium produced a decrease in magnitude of the movements; they were, to some extent, restored by the addition of calcium (fig. 5). The presence of calcium up to 500 mg./litre had little effect on motility.

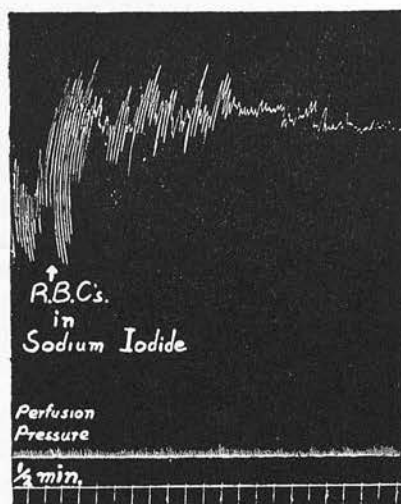


FIG. 4.—The effect of perfusing the ileum with red cells suspended in isotonic sodium iodide.

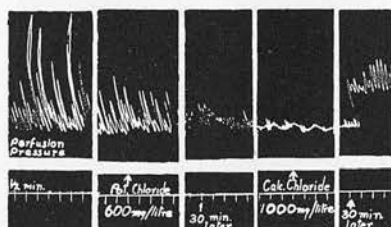


FIG. 5.—The effect of large doses of potassium on bowel motility and the antagonistic action of calcium.

### *Propulsion Experiments.*

Occasionally, during the course of the experiments, the recording balloon was found to have travelled to the aboral end of the perfused loop. Since earlier experiments showed that large boluses of cotton-wool and paraffin were only moved to and fro in the perfused loop with an overall oral progression [Alexander, 1949], this new phenomenon caused particular interest. The speed of this form of propulsion is illustrated by the following experiment:—

Loop of Jejunum 75 cm. long.

Trial number . . .	1	2	3	4	5	6
Time to traverse . .	15	20	23	20	15	15 seconds

Various causes of this rapid propulsion were considered, and it was thought that the degree of distension of the recording balloon was the

most probable. This was investigated, and it was found that, although increasing the pressure in the balloon increased the magnitude of the contractions, propulsion was not necessarily produced. This effect is shown in fig. 6.

The possibility of the property of rapid propulsion being limited to a particular part of the bowel was considered. The perfusion apparatus was arranged to perfuse two loops of bowel simultaneously. A segment of intestine was taken immediately adjacent to the duodenum, together with a second segment from the middle of the ileum; the two loops

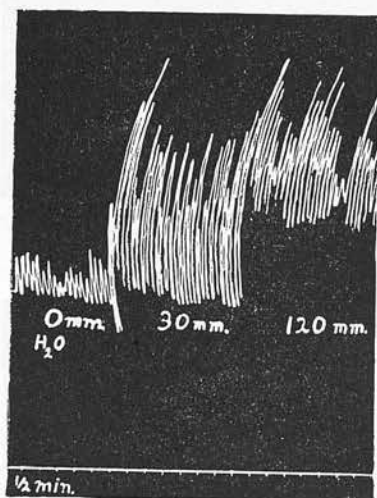


FIG. 6.—The effect of increased pressures in the recording balloon on the magnitude of the contractions.

were perfused so that their venous outflows were approximately equal, and tested for rapid propulsion by the same balloon distended to the same pressure. The results of these experiments showed that rapid propulsion could be demonstrated in loops from different parts of the bowel, but possibly more easily in the upper jejunum. It must be emphasised that rapid propulsion was only shown by about one-third of all guts examined.

An attempt was made to study the nature of the stimulus to the mucosa. The balloon was distended until it nearly filled the lumen. The distended balloon was rotated and rubbed to and fro, but this procedure did not cause the rapid propulsion more frequently than simply distending the balloon.

*Appearance of the Bowel during Rapid Propulsion.*—The impression gained from observing the perfused ileum whilst the balloon was traversing the lumen, was of the longitudinal muscles pulling the bowel over the balloon. Further, when the balloon was about the middle of

the loop of bowel, that part of the bowel through which the balloon had passed showed marked activity, the part caudal to the balloon was quite relaxed and inactive. Kymographic records taken while the balloon was passing along the bowel were in no way different from those taken from loops which did not show rapid propulsion. The rhythmic contraction of bowels showing rapid propulsion were commonly smaller than those not showing this property.

*The Effect of Drugs on Rapid Propulsion.*—The rapid propelling mechanism was completely abolished by nicotine (1 mg./100 ml. blood). It could not be induced by acetylcholine, carbaminoyl choline, eserine, pilocarpine or histamine.

#### DISCUSSION.

In the earlier experiments [Alexander, 1949] the gut only showed movements when oxygenated red cells were present in the perfusion fluid. The experiments recorded here provide evidence that the oxygen has an important action on the movements, and make it likely that the effect of the red cells is due to the oxygen which they carry. The experiments showed that the most marked effect of diminishing the oxygen supply to the gut was to decrease the magnitude of the rhythmic contractions. The rate of contraction was unaffected.

Of the other constituents of the perfusion fluid sodium seemed to be the most important—in its absence the gut showed no movements. Chloride, on the other hand, could be replaced by bromide, phosphate or sulphate, but not iodide.

*The Fast Propelling Mechanism.*—In the propulsion experiments very light balloons were used, and it was felt that they more nearly resembled the normal fluid contents of the ileum of the horse than did the lumps of cotton-wool used in the previous experiments. This mechanism resembled the peristaltic reflex of Bayliss and Starling [1899], the bowel above the balloon contracting and that below being inactive. However, the reflex these authors obtained by pinching above and below a recording balloon in the lumen of a dog's intestine was rarely demonstrable on the perfused horse ileum.

The main difficulty in studying this rapid propulsion was that only about one in three of the intestines studied showed this property. There was no difficulty in repeatedly demonstrating the phenomenon in those intestines showing rapid propulsion. An attempt was made, therefore, to find the reason for its absence from so many preparations.

The details of the experimental technique were reviewed to see whether any deviation was occurring in different experiments, but did not yield any result. Since Alvarez [1937] showed that anoxæmia usually stopped the peristaltic rush in the rabbit, and as the horse intestine is especially sensitive to anoxæmia, this factor was carefully



considered. Again, when two loops were perfused simultaneously from the same reservoir of blood, one loop showed rapid propulsion whilst the other did not. As the nature of the material imposed a delay between the time of slaughter and the beginning of the experiment usually of about two hours, this factor required some consideration. However, it was found that rapid propulsion occurred just as often in loops of bowel perfused three to four hours after slaughter as in those perfused within one hour.

When the pressure in the recording balloon was raised, the magnitude of the contractions was increased. This effect was similar to the response of the guinea-pig's ileum to increased pressure, as described by Trendelenburg [1917]. However, the increase in magnitude of the contractions of the horse did not produce propulsion.

The rapid propulsion was reminiscent of Bayliss and Starling's [1899] "Law of the Intestine". However, a mechanism as difficult to elicit as that described here can hardly be called a law. There has been a great deal of controversy about the part played by this myenteric reflex in the transport of food residues along the alimentary tract. Alvarez [1924] was unable to find evidence of the "Law of the Intestine" in the rabbit, and Bayliss and Starling [1901] had difficulty in demonstrating this "law" in the cat. It would be interesting to discover the reason for this variation between different species in the facility with which the myenteric reflex can be elicited.

In view of the experience of Garry [1933] it was hoped that a rubbing stimulus to the mucosa would induce rapid propulsion. This stimulus was, however, ineffective, although Garry found it a more certain stimulus to defecation than distension.

It seemed, therefore, from experiments described here and from earlier work, that at least two mechanisms were involved in the aboral propulsion of ingesta along the horse's ileum. Small particles were propelled slowly along the gut [Alexander, 1949], whereas the rapid propulsion described here was so different in speed and appearance as to suggest a different mechanism. Since both types of propulsion have been demonstrated in isolated preparations, they must have been produced by structures present in the bowel wall. It is important to conduct further experiments on the intact animal to see whether they can be shown under more physiological conditions.

#### SUMMARY.

1. The magnitude of the rhythmic contractions of the perfused ileum varied with the supply of oxygen.
2. Isotonic sodium chloride solution was as good as Tyrode's solution for suspension of the red cells in the perfusion fluid.

3. Variation of the potassium and calcium content of the perfusion fluid had little effect on motility. Sodium ions had a specific function in maintaining motility.

4. The chloride ions in the perfusion fluid were replaced by bromide, phosphate and sulphate ions without affecting motility. Iodide ions were toxic.

5. The ileum perfused with a suspension of blood cells propelled light balloons at rates of 1-4 cm./sec. in an aboral direction.

#### ACKNOWLEDGMENTS.

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# CONTENTS

	PAGE
ALEXANDER, FRANK. Factors Influencing the Motility of the Perfused Horse Intestine . . . . .	I
LORAINÉ, J. A. The Renal Clearance of Chorionic Gonadotrophin in Normal and Pathological Pregnancy . . . . .	II
O'CONNOR, W. J. The Rôle of the Neurohypophysis of the Dog in Determining Urinary Changes, and the Antidiuretic Activity of Urine, following the Administration of Sodium Chloride or Urea . . . . .	21
ADAM, H. M., HUNTER, R. B., and KINNEAR, T. W. G. Observations on the Urinary Excretion of Histamine in Urticaria . . . . .	49

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EXPERIMENTS ON THE HORSE STOMACH. By FRANK  
ALEXANDER. From the Rowett Research Institute, Bucksburn,  
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*(Issued June 1951)*

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# QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY

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EXPERIMENTS ON THE HORSE STOMACH. By FRANK  
ALEXANDER. From the Rowett Research Institute, Bucksburn,  
Aberdeenshire.

(Received for publication 10th October 1950.)

THE stomach of the horse presents several anatomical peculiarities, and these have aroused a great deal of speculation about its function, unfortunately not always founded on experimental evidence. The earliest observations on the horse's stomach were made by Colin [1886], who experimented on the stomachs of freshly killed horses and found that it was impossible to force the gastric contents through the cardiac sphincter by increasing intragastric pressure. Attempts to study the stomach in the living animal were made by Schalk and Amadon [1921], who were particularly interested in the effects of feeding and fasting on gastric mobility as recorded by a balloon attached to the end of a stomach tube. They had difficulty in securing consistent records and in interpreting their findings, but described "hunger contractions" in the fasting animal. In the experiments described here the behaviour of isolated strips and of the excised horse stomach has been studied, together with records of the pressure changes in the stomach of normal horses and the effect of various humoral agents on these preparations.

*Anatomical Considerations.*—The horse's stomach accounts for only 12.5 per cent. of the total capacity of the alimentary tract [Colin, 1886]. It is situated high up in the abdomen under the 9th–16th ribs, and is compressed between the diaphragm and large colon. The pyloric outlet runs upward and forward, forming an acute angle with the cardia, which has a well-developed sphincter muscle. Nearly two-fifths of its surface is covered with stratified squamous epithelium [Sisson, 1921].

#### METHODS.

*Muscle Strips.*—Segments of stomach from freshly killed horses were stored in cooled Tyrode's solution. Strips  $1 \times 5$  cm. were cut from these and suspended in Tyrode's solution at  $38^{\circ}$  C. in an isolated organ bath. The recording technique and method of testing the drug effects were the same as those used on the horse's gut [Alexander, 1949].

*Excised Stomach.*—The entire stomach was taken from a freshly killed horse and suspended in Tyrode's solution at  $38^{\circ}$  C. in a bath of 40 litres capacity. Intragastric and intraduodenal pressures were recorded with McDowall volume recorders. In other experiments the stomach was perfused with a suspension



of red blood cells in Tyrode, using a technique similar to that used on the ileum [Alexander, 1949].

*Unanæsthetized Horse.*—Most of these experiments were made on four ponies, two aged and two under 3 years old. They weighed between 150–200 kg. The animals were trained to stand quietly in stocks with a tube in position passed into the stomach by the nasopharyngeal route. Three types of stomach tube were used: a standard white rubber tube 13.5 mm. outside diameter, a smooth rubber tube 6.5 mm. diameter, and stiff polythene tubing 3.0 mm. diameter. It was not difficult to pass the standard tube, but it was necessary to reinforce the narrower tubes with a wire in the lumen. A balloon was fixed to the end of the tube which passed into the stomach, and the other end was connected to a water manometer. Respiratory movements were recorded by means of a tambour connected to a length of corrugated rubber tubing encircling the body about the level of the 14th thoracic vertebra. Aqueous solutions of the drugs were injected either intravenously into the jugular vein or subcutaneously. Fresh solutions of histamine acid phosphate were prepared for each experiment, and the preparations of posterior pituitary (Burroughs Wellcome) contained 10 units per ml. The effect of each drug was tested at least four times on each pony, with an interval of at least four days between each test.

### RESULTS.

Strips of muscle from the body and saccus cæcus of the stomach showed strong, slow, rhythmic contractions. Strips from the pyloric part showed faster contractions. The effects of adrenaline and acetylcholine on these muscle strips are shown in Table I.

TABLE I.—THE EFFECT OF DRUGS ON MUSCLE STRIPS FROM THE HORSE'S STOMACH.

Region.	Drug.	Effect.
Saccus cæcus . . . .	Adrenaline	Inhibited contractions
	Acetylcholine	Raised tone
Body . . . . .	Adrenaline	Inhibited contractions
	Acetylcholine	Raised tone
Pylorus . . . . .	Adrenaline	Inhibited contractions
	Acetylcholine	Restored contractions
Lesser curvature . .	Adrenaline	Raised tone
	Acetylcholine	Raised tone

*Excised Stomach.*—After some difficulty very feeble contractions were obtained from the excised stomach suspended in Tyrode (fig. 1). They were never strong enough to propel material through the pylorus.

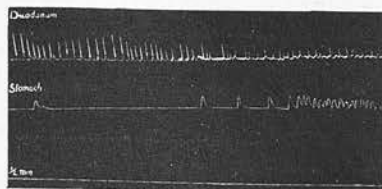


FIG. 1.—Contractions of the excised stomach suspended in Tyrode.

Acidulation of the contents did not cause emptying, although Cannon [1911] found that the excised cat's stomach, treated in this way, expelled its contents. Squeezing the stomach manually after ligature of the pylorus produced a rupture of the stomach, for the cardiac sphincter remained firmly closed and no food passed through it. This observation confirmed the original one of Colin [1886].

Perfusion of the gastric arteries with oxygenated red cells suspended in Tyrode failed to produce visible movements of the stomach.

### *The Unanæsthetized Horse.*

*The Effect of Different Sizes of Stomach Tubes.*—Since it was relatively simple to pass a standard stomach tube into the stomach, the first experiments were carried out with this tube. Records obtained by this method only showed slow tonus waves upon which were imposed the respiratory movements (fig. 2). It was difficult to understand how the

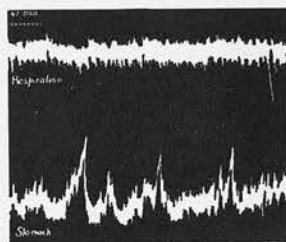


FIG. 2.—Intragastric pressure recorded with a standard stomach tube.

small changes in intragastric pressure represented by the tonus waves could be sufficient to push the stomach contents through the pylorus. The possibility of gastric contractions being influenced by swallowing was considered, and, in order to study this, it was necessary to devise a tube which could remain in place and yet permit the horse to eat. For this purpose a stiff polythene tube 3 mm. wide was passed through the nose and swallowed naturally by the pony. The records obtained with this tube differed completely from any obtained with the standard stomach tube, and consisted of powerful contractions unrelated to the respiratory movements (fig. 3, A and B). Their frequency of contraction was variable even with the same animal and varied from 3 to 8 per minute. Passing a standard stomach tube into the œsophagus completely inhibited these contractions (fig. 4) and explained the failure to record them with the standard tube. However, polythene tubes were too delicate for daily use, and rubber tubes, not more than 6.5 mm. wide, were used which gave satisfactory records with most horses.

*Effect of Fasting and Feeding.*—Fasting a horse 12–24 hours usually produced a quiescent stomach, the movements being a reflection of the respiratory movements (fig. 3, C). In some animals this period

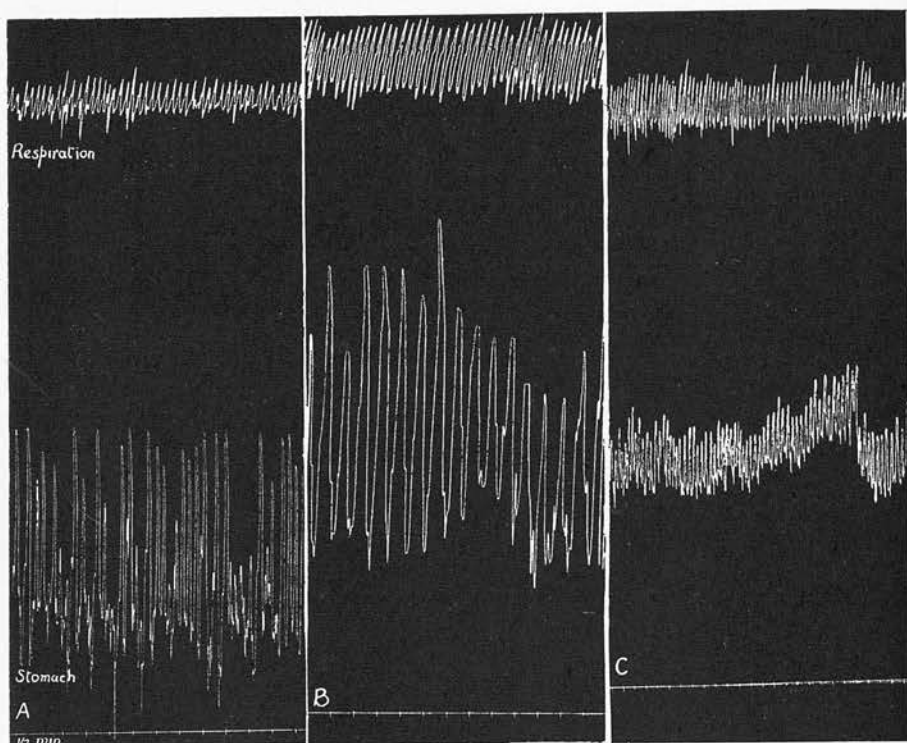


FIG. 3.—A and B. Contractions of the stomach after feeding recorded with a narrow tube. C. The same animal after a 24-hour fast.

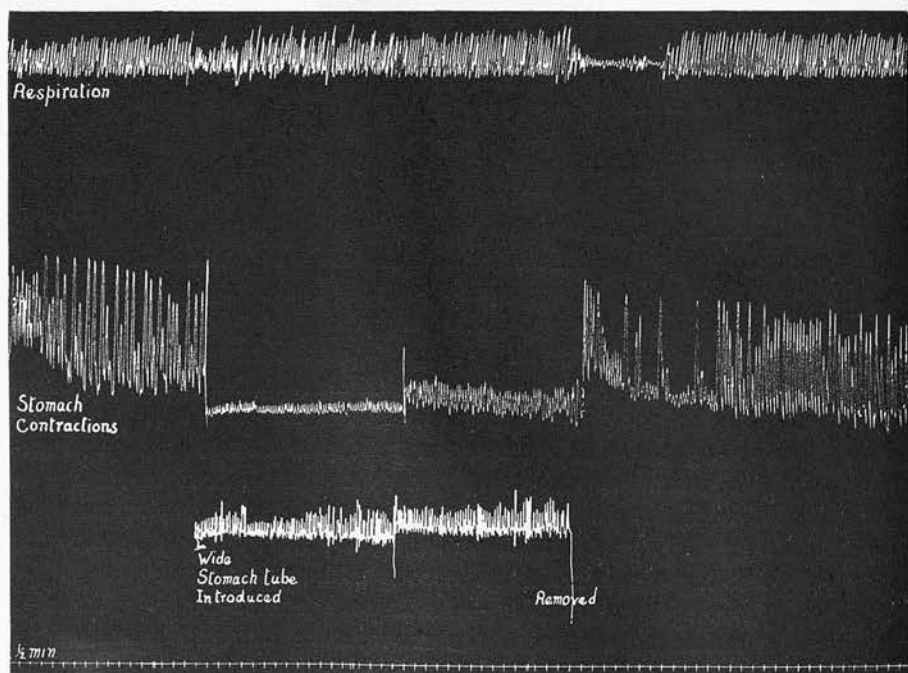


FIG. 4.—The inhibitory effect of standard stomach tube passed into the œsophagus. Simultaneous records from a narrow polythene tube and from the standard stomach tube are shown during the period of inhibition.

of quiescence was followed by intermittent periods of activity and quiescence. The intermittent periods of activity appeared to be those referred to by Schalk and Amadon [1921] as "hunger contractions." They were not inhibited by feeding (fig. 5). Feeding an animal whose stomach was quiescent induced contractions, but these ceased when feeding stopped and were resumed after a delay of from  $\frac{1}{2}$ –5 hours.

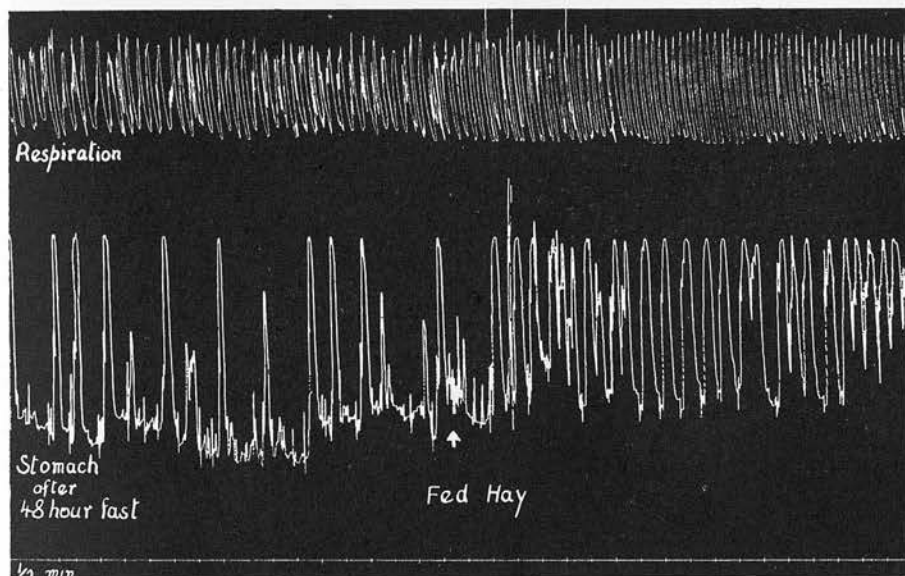


FIG. 5.—Failure of feeding to inhibit contractions of the fasting stomach.

*Effect of Drugs.*—In all animals adrenaline completely abolished stomach contractions for a few minutes (fig. 6), while atropine on injection stopped the stomach contractions for several hours (fig. 7).

Carbaminoylcholine given to a horse whose stomach was quiescent did not induce contractions, neither did it produce any change in the record when the stomach showed normal activity. In each instance the dose was sufficient to produce copious salivation, defæcation and colic, and these effects were stopped by injecting atropine.

Histamine, injected intravenously in animals whose stomach was quiescent, induced one or two contractions coincident with the occurrence of colic or of defæcation. When given to a horse showing normal gastric contractions it caused a small increase in the rate of contraction and a rise in tone (fig. 8).

The injection of posterior pituitary extract caused a slight increase in the contraction rate of the active stomach and diminished tonus changes in the quiescent stomach. The dose in each instance was sufficient to affect the pulse and respirations and produce colic and defæcation.

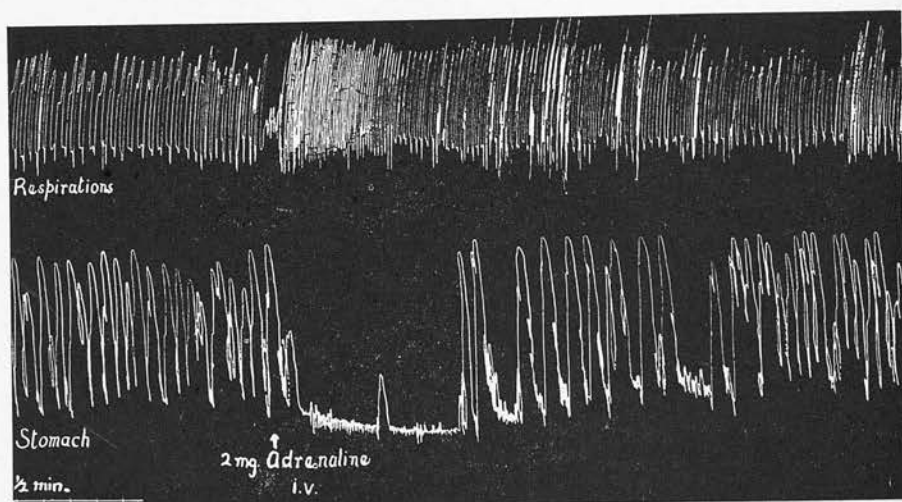


FIG. 6.—Effect of adrenaline on stomach contractions.

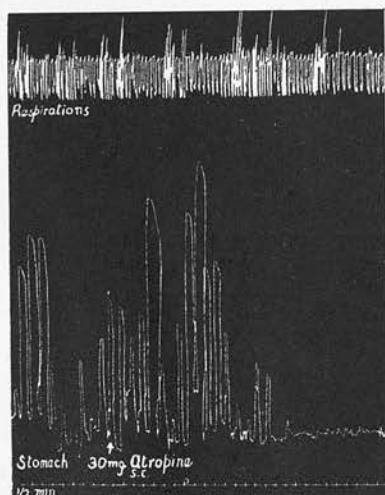


FIG. 7.—Effect of atropine on stomach contractions.

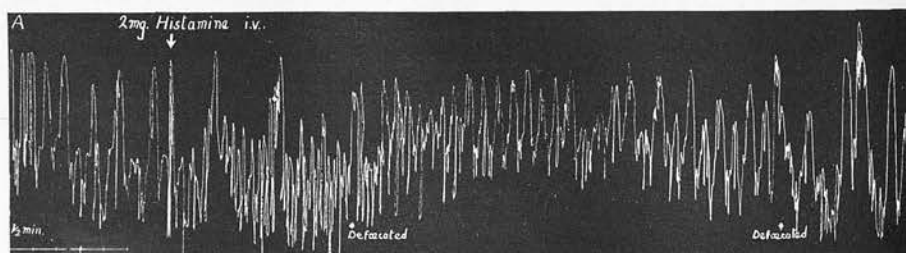


FIG. 8.—Effect of histamine on the contracting stomach.



## DISCUSSION.

Since strips of muscle cut from the horse's stomach and suspended in Tyrode exhibited strong contractions and gave typical responses to adrenaline and acetylcholine, it was difficult to understand why the whole organ, similarly suspended, could not propel its contents through the pylorus. The failure of the excised horse stomach to exhibit propulsive movements cannot necessarily be explained by the artificial nature of the preparation, since Hofmeister and Schütz [1886] found that the motility of the excised stomach of the dog was similar to that of the intact animal, and this was confirmed for the denervated rabbit stomach by Auer [1908]. Since an adequate supply of oxygen was necessary for the excised horse ileum to show propulsive movements [Alexander, 1949, 1950], similar conditions were provided for the stomach; but even these measures failed to produce propulsion. It seemed probable, therefore, that the mechanism responsible for emptying the horse's stomach depended upon its connections with the central nervous system or the integrity of the alimentary tract. In this respect the stomach was similar to the small colon which, even when perfused with oxygenated red cells, failed to propel its contents [Alexander, 1949].

It was therefore necessary to study gastric motility in the living horse and determine some of the factors governing it. As far as could be ascertained, the only workers to undertake such a study have been Schalk and Amadon [1921], and a technique similar to theirs was adopted for the present work. The first experiments were made with stomach tubes used in veterinary practice, as these could be passed without difficulty and were sufficiently rigid to push into the stomach. However, records obtained in this way showed only weak tonus waves and reflected respiratory movements which seemed inadequate to empty the stomach. Nevertheless, these records are similar to some of the tracings published by Schalk and Amadon [1921], who, unfortunately, did not make simultaneous records of intragastric pressure and respiratory movements.

It is clear, however, that records such as these were due to the inhibitory effect of a wide tube in the oesophagus, and when this was appreciated and overcome, movements were obtained not respiratory in origin and very different from any published by Schalk and Amadon. The existence of a similar inhibitory reflex in the dog has been shown by Quigley, Bavor, Read and Brofman [1943].

Although the fasting horse showed intermittent gastric contractions, an observation in agreement with that of Schalk and Amadon [1921], these contractions were not inhibited by food and therefore did not behave as "hunger contractions" in man [Carlson, 1912]. However, further work has shown that even in man hunger sensations are not



always accompanied by strong gastric contractions [Wolf and Wolff, 1947]. Moreover, "hunger contractions" in the rabbit [Rogers, 1915] and guinea-pig [King and Connet, 1915] are not inhibited by feeding.

Pharmacological studies on the stomach of the unanæsthetized horse showed that gastric contractions were easy to inhibit but hard to stimulate. The inhibition produced by atropine and adrenaline was similar to the effect these drugs produce on man [Wolf and Wolff, 1947]. In addition, the failure of carbaminoylcholine to induce contractions was similar to the lack of response of the human stomach to the related drug acetyl- $\beta$ -methylcholine [Wolf and Wolff, 1947]. The main difference noted was the failure of the horse's stomach to respond to the injection of posterior pituitary extract, although Wolf and Wolff found a marked increase in human gastric contractions following the administration of this drug.

These experiments have shown that gastric contractions were not produced in excised stomach even when perfused with blood, or in the intact animal by injecting the more common humoral agents. The necessary stimulus for gastric contractions must therefore depend upon the integrity of the digestive tract, the central nervous system, or on some product of digestion. Further experiments are necessary to analyse these factors and, in particular, to investigate the importance of the central nervous system in relation to gastric contraction.

#### SUMMARY.

1. Strips of muscle from the saccus cæcus of the horse's stomach showed slow contractions, and strips from the pyloric part faster contractions. Both were inhibited by adrenaline and stimulated with acetylcholine.

2. The excised stomach showed only feeble contractions even when perfused with blood.

3. Records of stomach contractions in the unanæsthetized horse were secured by using stomach tubes of narrow diameter.

4. Introducing a wide diameter stomach tube into the œsophagus inhibited stomach contractions.

5. Feeding induced contractions, and fasting for 12-24 hours caused them to stop or become intermittent. Intermittent contractions were not inhibited by feeding.

6. Stomach contractions were inhibited by adrenaline and atropine. Histamine, carbaminoylcholine and posterior pituitary extract had little action on the actively contracting or on the quiescent stomach.

## ACKNOWLEDGMENTS.

The preliminary part of this work was carried out at the Royal (Dick) Veterinary College whilst holding a grant from the Agricultural Research Council. I am indebted to the Council for the grant and to Professor W. M. Mitchell for laboratory facilities.

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# CONTENTS

	PAGE
GRIBBLE, M. de G., and PETERS, R. A. The Influence of Thyroidectomy on Post-burn N Loss in Rats . . . . .	119
BEKKUM, D. W. van, and PETERS, R. A. Observations upon a Change in an Enzymatic Process in Burns . . . . .	127
ALEXANDER, FRANK. Experiments on the Horse Stomach . . . . .	139
DUKE, H. N., PICKFORD, M., and WATT, J. A. The Antidiuretic Action of Morphine: Its Site and Mode of Action in the Hypothalamus of the Dog . . . . .	149
HEBB, CATHERINE O., and LINZELL, J. L. Some Conditions Affecting the Blood Flow through the Perfused Mammary Gland, with special reference to the Action of Adrenaline . . . . .	159
PEARCE, J. W., and WHITTERIDGE, D. The Relation of Pulmonary Arterial Pressure Variations to the Activity of Afferent Pulmonary Vascular Fibres . . . . .	177

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**The preparation of Biebl loops and Thiry-Vella fistulae of the ileum of the horse.** By F. ALEXANDER. *Rowett Research Institute, Bucksburn, Aberdeenshire*

The principal reason for the lack of knowledge about the digestive processes in the horse has been the surgical problem of making chronic preparations giving access to the intestine. A technique for fistulating the large colon has already been described (Alexander & Donald, 1949). An adaptation of the Biebl loop and Thiry-Vella fistula to the horse ileum has been developed.

The ileum was approached through the flank, the operative site being limited cranially by the 18th rib and caudally by a line parallel to the last rib and immediately in front of the coxal tuberosity.

Anaesthesia was induced with sodium pentobarbital (10 mg./kg.) and maintained by subsequent doses of 2 mg./kg. The last six intercostal nerves were blocked with a 1/1000 nupercaine solution.

In the preparation of a Biebl loop a fold of skin was defined about 5 in. wide and 9 in. long running in the direction of the fibres of the internal oblique muscle. This skin flap was freed from the body wall except at the two ends, and the abdomen entered through an incision in the middle of the exposed fascial surface, cutting across the external oblique and dividing the fibres of the internal oblique and transverse abdominal muscles by blunt dissection. A loop of ileum was brought into the wound, the avascular part of its mesentery incised and the peritoneum and the muscles of the abdominal wall joined with silk sutures through this incision, leaving a space at each end so that the lumen of the ileum was not occluded. The exteriorized ileum was enclosed within the bipediced skin flap. The skin of the abdominal wall was closed beneath the loop so formed.

For the preparation of a Thiry-Vella loop the abdomen was approached through the flank, the abdominal muscles being separated by blunt dissection in the direction of their fibres. A loop of ileum, about 50 cm. long and supplied by at least one intestinal artery, was sectioned between clamps. Two stab incisions one above the other were made about 3 in. from the main wound. The ends of the ileal loop were grasped with long clamps passed through the stab wounds and thus drawn through them. It was important to allow at least 2 in. of ileum to protrude from the stab wound before securing with four nylon sutures. Continuity of the ileum was restored by an end-to-end anastomosis using linen sutures. The muscles and peritoneum were re-united with silk and the skin wound with nylon sutures.

I am indebted to Prof. D. M. Douglas for advice on the preparation of Biebl loops.

REFERENCE

Alexander, F. & Donald, D. E. (1949). *J. comp. Path.* 59, 127.

SOME FUNCTIONS OF THE LARGE INTESTINE  
OF THE HORSE

By  
FRANK ALEXANDER

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SOME FUNCTIONS OF THE LARGE INTESTINE OF THE HORSE. By FRANK ALEXANDER.<sup>1</sup> From the Rowett Research Institute, Bucksburn, Aberdeenshire.

(Received for publication 14th July 1952.)

IN a review of the movements of the large intestine, Garry [1934] emphasized the importance of structure in relation to function of the colon and suggested that the human colon was nearer to the herbivorous than to the carnivorous type. For the most part, however, colon function has been studied in dogs or in men with colonic disease, and apart from the work of Elliott and Barclay-Smith [1904] the herbivorous colon has attracted little attention. The horse offers an example of a herbivore in which the cæcum and colon are greatly enlarged, presumably to provide a fermentation chamber for the digestion of cellulose. Surgical techniques for inserting fistulæ into the horse's large intestine have been developed in order to study these organs, and three aspects of their physiology are discussed in this paper: (1) the spontaneous pressure changes within the lumen of cæcum and colon, (2) the action of certain drugs upon movements so recorded, and (3) the fermentative properties of the contents of these parts of the gut.

METHODS.

*Surgical.*—The original procedure of Alexander and Donald [1949] was adequate for a single fistula; it required modification when several fistulæ were needed. Anæsthesia was induced by the intravenous injection of sodium pentobarbitone. After blocking the last six intercostal nerves with a 1/1000 Nupercaine solution, a skin incision about 8 in. long, and 3–4 in. from and parallel to the last rib, was made. The abdominal muscles were divided by blunt dissection and the exposed peritoneum cut with scissors between Allis forceps. A segment of the right dorsal colon about 6 in. long was isolated between clamps, and in an area between two tænia an elliptical piece of bowel wall about 3 in. in its long axis was defined by a purse-string suture of thick silk. Within this an incision about 3 in. long was made, the inner flange of the cannula introduced, and the ligature tied tightly around it. A reinforcing suture was then applied, making certain all mucous membrane was buried. The cannula was brought to the outside through a stab wound about 2 in. from the main incision and secured by a rubber disc and

<sup>1</sup> Now at the Department of Veterinary Pharmacology, Royal (Dick) School of Veterinary Studies, Edinburgh University, Edinburgh.



screw collar. When cannulae were inserted into three different parts of the large intestine at one operation, it was important to cannulate the dorsal before the ventral colon, and the caecum last. Peritoneum and muscles were sutured with silk and the skin with nylon.

A satisfactory cannula was made from "Alkathene" tubing and rubber filter discs (fig. 1). A thread was cut on one end of a piece of tubing 6 in. long and the other end was flanged. Two rubber discs were stuck together and pushed up to the flanged end to form the inner collar. The outer collar was formed by a single rubber disc kept in place by a lock nut and screw-cap.

*Recording.*—Kymographic records of pressure changes were made by inserting balloons connected to water manometers into the lumen of the organ. Segments of condom 3 in. long, inflated to about 15 cm. water pressure, formed the balloons, and where three records were made simultaneously the water manometers were connected to tambours, otherwise frontal writing points were used.

The drugs to be tested for their action were injected intravenously; the pituitary extract of Parke Davis & Co., "Pituitrin", was used, and solutions of histamine-acid-phosphate were freshly prepared for each experiment. The effects of drugs, feeding and of withholding food were tested at least four times on each animal, with a similar result in every instance. Two ponies with fistulae in caecum, right ventral and right dorsal colon, one pony with fistulae in the right dorsal and right ventral colons, and four ponies with fistulae in either caecum or right ventral colon, were used for these experiments.

Total volatile fatty acids were estimated by titrating the steam distillate of acidified strained colon contents with 0.02N NaOH under CO<sub>2</sub>-free conditions, using phenol red as indicator. Distillates were neutralized, concentrated by heat and extracted with 5 per cent. butanol in chloroform [Elsden, 1946]. Separation of the fatty acids was carried out by the chromatographic method of Baldwin, Moyle and Scarisbrick [1948]. The rate of cellulose breakdown was determined by the modifications of the method of Hofflund, Quin and Clark [1948], described by Balch and Johnson [1950], and consisted of measuring the loss in weight of cotton-threads suspended for various intervals within the different parts of the large intestine.

Except where it is stated to the contrary, the animals were fed at 7 a.m., 11 a.m. and 4 p.m. daily, a total of 6 lb. hay and 3 lb. oats being given to each pony daily.

## RESULTS.

### *Normal Motility.*

*Movements of the Large Intestine.*—Kymographic recordings were usually made during the day. On three occasions they were made

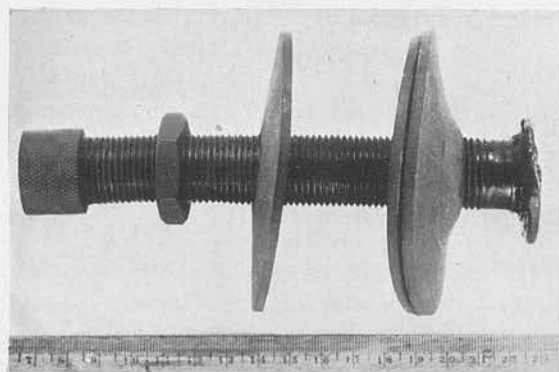


FIG. 1.—An "Alkathene" cannula. Scale in cm.

over a 12-hour period. The movements recorded were of three types: (a) large contractions which constituted the greatest pressure changes recorded; (b) smaller movements of a higher frequency which did not appear to be co-ordinated between the different parts of the large intestine and which were observed oftenest in the dorsal colon; (c) tonus changes (fig. 2). Withholding food for 24–48 hours reduced the number of contractions, particularly in the cæcum and ventral colon when type (b) pressure changes were practically abolished. Tonus changes were also reduced (fig. 2). Feeding a pony not previously fasting, however, did not alter the frequency or strength of the contractions of any of the three parts of the large intestine.

The movements of the cæcum and colon were also observed when these structures were exposed at operation, since opening the abdominal cavity did not have any marked inhibitory effect. The cæcum was generally very active, showing a rippling movement of the haustra and periodic, powerful contractions of the tænia.

*Absence of Antiperistalsis.*—Elliott and Barclay-Smith [1904] concluded from observations on the denervated large intestines of common laboratory herbivores (exposed under saline) that antiperistaltic propulsion of material from the proximal colon into the cæcum was a characteristic movement. In earlier work on the horse it was found that carbon granules made suitable markers for determining the rate of progress of food residues along the bowel [Alexander, 1946]; hence this method was adopted to see whether material from the right ventral colon moved orally to the cæcum, that is, in reverse to the normal direction. Six experiments were carried out in which carbon granules were put into the right ventral colon; samples of about 250 ml. were taken from the cæcum and dorsal colon at each subsequent hour and carefully searched for the presence of carbon granules. In no instance were carbon granules detected in the cæcum, although they always appeared in the right dorsal colon, often within 4 hours of administration. Antiperistaltic propulsion of carbon particles from the right dorsal to the right ventral colon was investigated, but again oralward movement was not observed. It was considered possible that colonic contents might be moved backwards to the cæcum when the animal had not fed for 24–48 hours, but this was not so, since no carbon was detected in the cæcum in samples taken up to 5 hours after administration.

*The Effect of Drugs.*—The responses to the injection of adrenaline, carbamylcholine, histamine and posterior pituitary extract were essentially the same as those previously recorded from the ileum of the horse [Alexander, 1952]. Adrenaline and posterior pituitary extract inhibited movements. Histamine caused an inhibition followed by increased motility, and carbamylcholine increased both frequency and magnitude of the contractions. The duration of the inhibitory effect of adrenaline, histamine and posterior pituitary, however, differed in the

three portions of the large intestine. Adrenaline inhibition persisted for longer periods and histamine inhibition for shorter periods in the dorsal colon than it did in the cæcum (figs. 3, 4, 5 and 6). Inhibition produced by posterior pituitary persisted for 15 minutes or more, and in ponies in which a portion of colonic mucous membrane had grown past the cannula and was exposed to view, it was observed that pallor followed the injection of this drug. Defæcation occurred occasionally after the injection of posterior pituitary extract.

*The Volatile Fatty Acid Concentration in Cæcum and Colon.*—The absorbed products of cellulose digestion in most herbivores, including the horse, appear to be the lower volatile fatty acids [Elsden, Hitchcock, Marshall and Phillipson, 1946]. The factors influencing the production of these substances in the ruminant have been given considerable attention [Phillipson, 1947], but so far they have not been investigated in the horse. This has been due in part to the difficulty of obtaining cæcal and colonic contents from the living animal, a difficulty which has now been overcome.

The concentration of volatile fatty acid fraction found in ponies cannulated in the manner described varied (a) with the diet, (b) with the interval after feeding, and (c) according to which part of the large intestine was being sampled. Table I shows an example of the variation

TABLE I.—TOTAL VOLATILE FATTY ACIDS AS MG. ACETIC ACID PER 100 ML. VENTRAL COLON FLUID OF SAME PONY ON DIFFERENT DIETS.

Time.	Grass.	Hay only.
9 a.m.	463	495
10 a.m.	469	496
11 a.m.	511	493
12 a.m.	422	476
1 p.m.	526	511
2 p.m.	508	469
3 p.m.	517	493
4 p.m.	514	469
Range over 6 expts.	402–636	312–552

through the day under normal feeding conditions, and the differences arising when the same animal is fed entirely on hay rather than on grass. This result was typical of six similar experiments. The concentration of fatty acids was slightly higher in the grass-fed animal compared with the same animal fed hay. The concentration differed between cæcum, right ventral and right dorsal colons, and the effect of withholding food was more evident in the cæcum and ventral colons than in the dorsal colon. An example is shown in fig. 7. In one pony out of three a marked increase was seen within 3 hours of giving a feed of hay; the withholding of food depressed the appetite of two of the ponies, which therefore failed to show this increase.

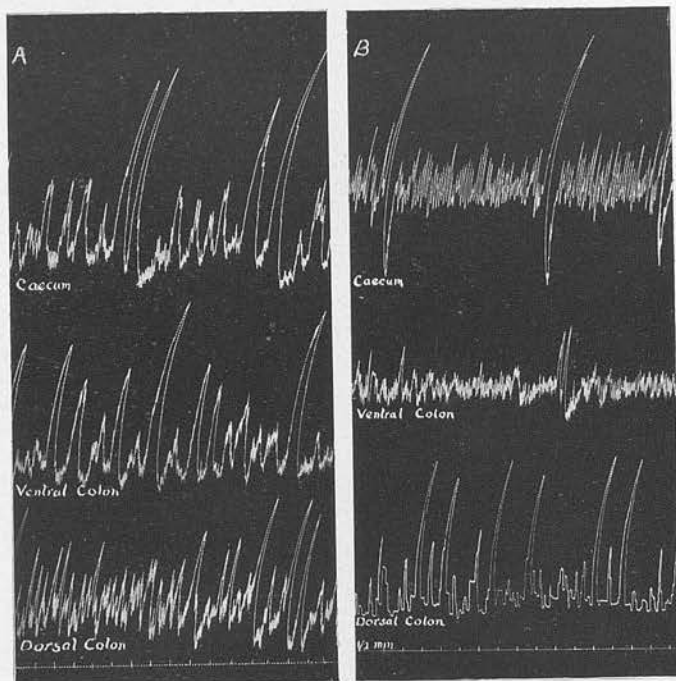


FIG. 2.—A. Contractions of the cæcum, ventral and dorsal colons in a normally fed animal.  
B. The same animal 48 hours after the last feed.

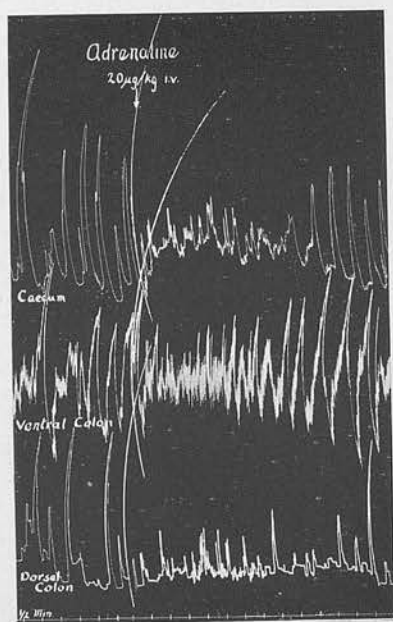


FIG. 3.—Effect of adrenaline on the movements of the large intestine.



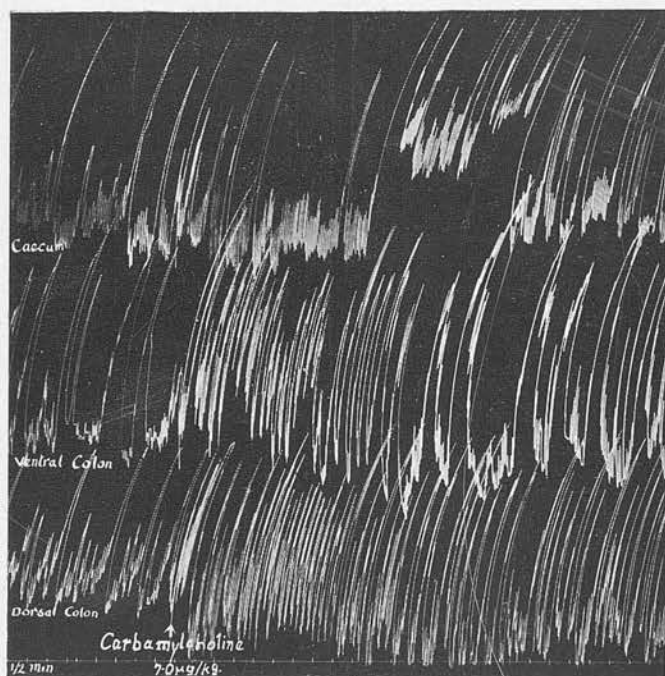


FIG. 4.—Effect of carbamylcholine on the movements of the large intestine.

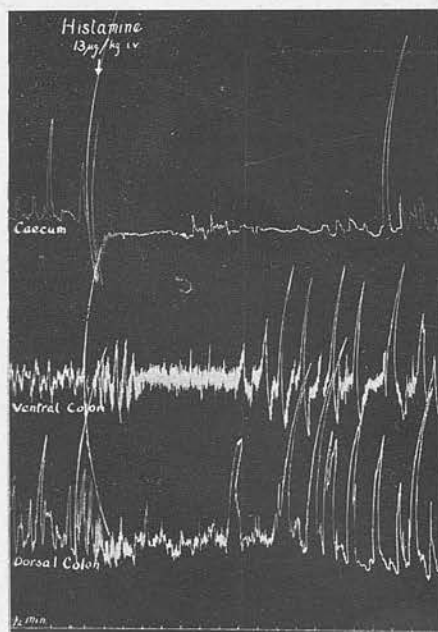


FIG. 5.—Effect of histamine on the movements of the large intestine.

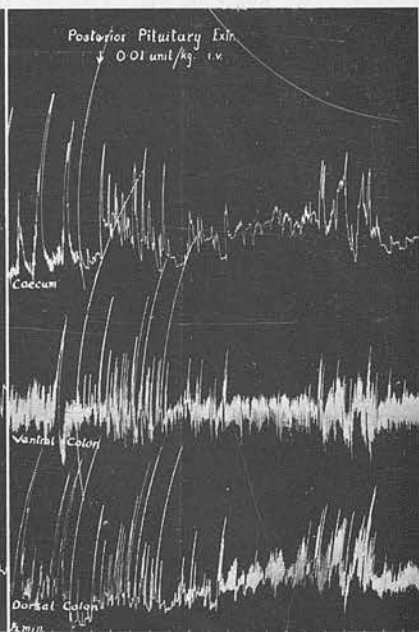


FIG. 6.—Effect of posterior pituitary extract on the movements of the large intestine.



TABLE II.—FATTY ACID CONCENTRATION OF GUT CONTENTS EXPRESSED AS MG. ACETIC ACID PER 100 ML. FLUID.

	Cæcum.	Colon ventral.	Colon dorsal.
48 hours after last feed	64.3	125.5	213.0
3   "       "       "	285.8	373.3	190.3
20   "       "       "	400.9	401.5	261.3

Although Elsdén *et al.* [1946] showed that the fatty acid mixture in the cæcum and colons of the horse consisted mainly of acetic, propionic and butyric acids, they made no distinction between the two parts of

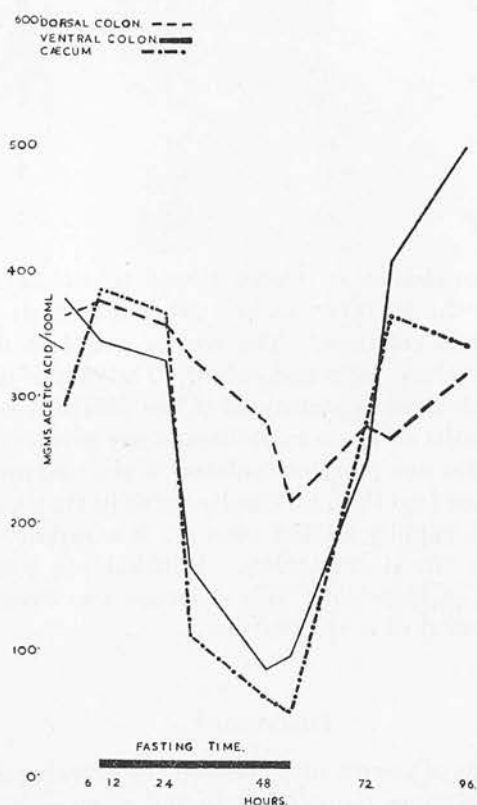


FIG. 7.—The effect of withholding food on the concentration of total volatile fatty acids (expressed as mg. acetic) in the cæcum, ventral and dorsal colons.

the large colon. Since the material in the dorsal colon had already been subjected to digestion and fermentation in the stomach, ileum, cæcum and ventral colon, it seemed possible that the fatty acid mixture might differ between the ventral and dorsal colons. Chromatographic separations of the fatty acids were carried out, and the mixture in the

ventral colon differed from that in the dorsal colon and contained more propionic acid. The results are shown in Table III.

TABLE III.—A COMPARISON OF THE FATTY ACIDS PRESENT IN THE VENTRAL AND DORSAL COLONS EXPRESSED AS A PERCENTAGE OF THE TOTAL CONCENTRATIONS.

Animal.	Region.	Per cent. total volatile fatty acid.			
		Acetic.	Propionic.	Butyric.	Higher.
Blackie	Ventral colon	68	24	6	2
	Dorsal    ,,	71	19	7	3
Blackie	Ventral    ,,	63	28	6	3
	Dorsal    ,,	67	13	12	8
Blackie	Ventral    ,,	69	23	6	2
	Dorsal    ,,	66	17	7	6
Judy	Ventral    ,,	66	25	6	2
	Dorsal    ,,	71	17	7	5
Tilly	Ventral    ,,	65	17	7	4

*The Rate of Breakdown of Cotton-thread (Cellulose).*—The greatest difference between the herbivorous and other mammals is the ability of the former to utilize cellulose. The site of cellulose digestion in the horse appears to be the cæcum and colon. The rate of disappearance of cotton-thread cellulose was determined in the different parts of the large intestine. The results of these experiments are given in Table IV, and are complete only for one pony cannulated in the cæcum and both parts of the colon. Taken together, all results agree in that the cotton-thread disintegrated most rapidly in the cæcum, less rapidly in the ventral colon and least in the dorsal colon. Withholding food for 24 hours decreased the rate of digestion: this decrease was even greater during the last 24-hour period of a 48-hour fast.

#### DISCUSSION.

The contractions of cæcum and colon of the horse were more frequent and appeared to be more powerful than the colon contractions of man [Grace, Wolf and Wolff, 1951] or the dog [Templeton and Lawson, 1931], although the progressive contractions described by the latter authors were recorded in the horse. Elliott and Barclay-Smith [1904] studied the movements of the denervated colon exposed under saline, and found that the characteristic activity of the herbivorous colon was to propel starch paste or similar material to and fro, from colon to cæcum and back. However, no evidence of antiperistalsis was obtained from observing the propulsion of carbon granules in the horse. This agreed with the

TABLE IV.—RATE OF DIGESTION OF COTTON-THREAD IN THE CÆCUM, DORSAL AND VENTRAL COLONS.

Pony.	Expt. no.	Per cent. cotton-thread digested.			Time in animal.
		Cæcum.	Ventral colon.	Dorsal colon.	
During normal feeding.					
Punch	1	..	0	0	4 hrs.
Blackie	1	..	0	0	} 8 hrs.
Tilly	1	..	0	..	
Punch	1	..	19	10	} 16 hrs.
Punch	2	..	24	6	
Blackie	1	..	36	9	
Tilly	1	..	5	..	
Judy	1	37	23	12	
Blackie	1	..	53	36	} 24 hrs.
Blackie	2	..	58	..	
Punch	1	..	22	19	
Tilly	1	..	28	..	
Judy	1	52	25	19	
Judy	2	76	42	24	
Judy	3	67	39	24	
Judy	4	66	49	27	
Judy	5	62	26	13	
Punch	1	..	100	100	} 48 hrs.
Tilly	1	..	100	..	
Blackie	1	..	100	65	
Blackie	2	..	100	100	

## After a 24-hour fast.

Judy	1	0	0	6	} 24 hrs.
Judy	2	0	0	28	
Judy	3	0	0	36	
Blackie	1	..	3	12	
Blackie	2	..	11	9	
Blackie	3	..	11	11	} 24 hrs.
Tilly	1	..	19	..	

Blackie. Fistulæ in ventral and dorsal colon.  
 Judy. Fistulæ in cæcum, ventral and dorsal colon.  
 Punch. Fistulæ in ventral and dorsal colon.  
 Tilly. Fistulæ in ventral colon.

work of Alvarez [1948], who could find no evidence of such activity in the rabbit.

The contractions of the large intestine were reminiscent in form of the large, slow contractions seen in the ileum [Alexander, 1952], and like them, the caecal and ventral colonic contractions decreased when food was withheld. However, the dorsal colon movements were unaffected by 48 hours' fasting and its fatty acid concentration was little changed. When the effects of the various humoral agents on caecal and colonic activity were compared with their effects on the horse ileum, it was noted that histamine and posterior pituitary extract inhibited movements in all these organs. Since isolated strips of large intestine were stimulated by histamine and unaffected by posterior pituitary [Alexander, 1949], the inhibition obtained in the intact animal could not be explained by the direct action of the drug on intestinal muscle. However, in the ileum it was shown [Alexander, 1952] that the inhibitory effect of these drugs was accompanied by a decrease in ileal temperature and by cyanosis of the mucosa of a Thiry-Vella loop. As the ileum was shown to be very sensitive to anoxia [Alexander, 1950], the inhibition of movement could be explained by a decreased ileal blood flow produced by histamine or posterior pituitary extract. The inhibition of caecal and colonic movements by the same drugs could also be due to a decreased blood flow through these organs.

Elsden and his colleagues [1946] found that large quantities of volatile fatty acids were present in the caecum and colon of the horse, and Barcroft, McAnally and Phillipson [1944] showed that these acids were absorbed from the organs in which they were produced. The development of a satisfactory fistulation technique made possible the extension of these observations by studying factors influencing fatty acid production. The volatile fatty acid production of the hay-fed pony was only slightly less than when the same animal was fed grass and oats, whereas the grass-fed sheep produced twice the amount of fatty acid than when fed hay alone [Phillipson, 1942]. A reason for this distinction may be that the more soluble carbohydrates are digested and absorbed in the ileum of the horse whilst in sheep all food is fermented in the rumen. Since the fatty acids in the caecum and colon decreased on withholding food and increased on feeding, some animals showing an increase within 3 hours, it seemed reasonable to conclude that these substances arose from fermentation within the large intestine. With regard to this rapid onset of fermentation, it is interesting to note that Ritzman and Benedict [1938], who were puzzled by the large heat increment that followed feeding in horses and cattle, rejected the explanation that it was due to the heat of fermentation and the rapid absorption of the soluble products of fermentation, as they supposed that considerable time had to elapse in the horse before the food reached the large intestine. The results described here showed that fermentation,

as judged by the production of volatile fatty acids, increased in the cæcum and colon very soon after feeding. Further, Alexander and Benzie [1951] demonstrated radiologically the rapid propulsion of barium through the stomach and small intestines of the grass-fed foal. The objections raised by Ritzman and Benedict [1938] to attributing the heat increment following feeding in the horse to fermentation are, therefore, no longer valid. The separation of the fatty acids showed that more propionic acid was produced in the ventral than in the dorsal colon, and more butyric and higher acids in the dorsal than in the ventral colon. The reason for this difference requires further investigation.

Digesta remain in the alimentary tract of the horse 15-48 hours [Alexander, 1946], whereas in cows the time is much greater; 80 per cent. of a marker was excreted between 70 and 90 hours after administration [Balch, 1950]. Since both species depend to a large extent upon the products of cellulose digestion, it was of interest to compare the rate of cotton-thread digestion in the cæcum and colon with the rate in the rumen. The rate of digestion in the ventral colon was similar to that found by the same method in the ventral sac of the rumen in cows given hay and concentrates [Balch and Johnson, 1950]: in the hay-fed cow, the rate of digestion in the ventral sac of the rumen was less than in any part of the horse colon. It appears, then, that the capacity of the horse to ferment cotton-thread cellulose is not inferior to that of the cow. The marked decrease in the digestion of cotton-thread when food was withheld is not easy to understand, and it may be that in the absence of readily available carbohydrates and nitrogen, and possibly other factors, the bacteria or other organisms responsible are depressed in numbers and activity and hence are unable to utilize it as a substrate.

#### SUMMARY.

1. Techniques are described for cannulating the cæcum, ventral and dorsal colon of the horse.

2. Cæcal and colonic movements consisted of irregular contractions of which three types were recognized. Withholding food caused reduction in frequency or cessation of all contractions of cæcum and ventral colon; the dorsal colon remained unaffected. No evidence was obtained of antiperistaltic movements, as judged by the movement of carbon granules.

3. Adrenaline inhibited and carbamylcholine increased the frequency of the contractions. Histamine and posterior pituitary extract inhibited movements.

4. The concentration of volatile fatty acid in the liquor of the cæcum and ventral colon was decreased by withholding food and increased by feeding. This effect was not marked in the dorsal colon. The proportion of propionic acid in the ventral colon was greater than in the dorsal colon.

5. The rate at which cotton-threads disintegrated when suspended in the cæcum, ventral and dorsal colon decreased in an oral-caudal direction. Withholding food greatly reduced the rate of disintegration.

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# THE EFFECTS OF SOME HUMORAL AGENTS ON THE HORSE ILEUM

BY

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## THE EFFECTS OF SOME HUMORAL AGENTS ON THE HORSE ILEUM

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(Received June 25, 1951)

In 1940 Tanaka and Ohkuho observed a marked contraction in isolated strips of horse ileum after the addition of adrenaline to the bath. This observation was confirmed and the effects of acetylcholine, histamine, and posterior pituitary extract studied in a similar manner (Alexander, 1949). It was found that acetylcholine and histamine caused a marked rise in tone and increased the magnitude of the rhythmic contractions, whereas the posterior pituitary extract was without effect. In other experiments with isolated blood-perfused ileum, similar responses were evoked with acetylcholine and histamine, whereas adrenaline inhibited motility and posterior pituitary extract caused a small contraction (Alexander, 1949). The experiments described here were designed to determine the response of the ileum in the living animal to adrenaline, carbamylcholine, histamine, and posterior pituitary extract.

### METHODS

Observations were made on three ponies with Biebl loops and one with a Thiry-Vella fistula. The surgical techniques involved in the preparation of Biebl and Thiry-Vella loops in the horse are described elsewhere (Alexander, 1951).

Recordings of movement in the Biebl loop were made with the double tambour system described by Douglas and Mann (1941) and from the Thiry-Vella loop by a balloon and water manometer.

Variations in the intestinal temperature were measured with two thermocouples and a galvanometer. The thermocouples were made from junctions of copper and eureka wire. The reference couple was placed in a vacuum flask containing water at about 37° C., along with a thermometer; the second couple was stitched through the exteriorized intestine so that the junction lay in or against the mucosa. A calibration curve for the galvanometer was constructed by plotting measured temperature differences between the two thermocouples against the galvanometer deflection produced. Thus it was possible, by reading simultaneously the galvanometer deflection and the temperature of the reference couple, to determine the temperature of the couple in the intestinal mucosa.

Aqueous solutions of adrenaline hydrochloride, carbamylcholine, histamine acid phosphate, and posterior pituitary extract were injected intravenously. The doses given were the least which consistently produced a measurable response; doubling this dose produced a similar but more lasting effect (Table I). It was considered unfair to subject the animals to undue distress by further increase of dosage. The effect of each drug was tested at least four times on each pony.

TABLE I

The effects on intestinal movements and temperature of adrenaline, carbamylcholine, histamine, posterior pituitary extract, and isotonic saline

Drug	Dose	Effect		
		Motility	Tone	Temperature
Adrenaline .. ..	10 $\mu$ g./kg.	Inhibited	Slight increase	Decreased
Carbamylcholine ..	20 $\mu$ g./kg.	Increased or made irregular	Marked increase	Unaffected or decreased
Histamine .. ..	20 $\mu$ g./kg.	Decreased	Marked increase	Decreased
Posterior pituitary extract	0.5 units/kg.	Decreased	Unchanged	Decreased
Isotonic saline .. ..	5 ml.	Unchanged	Unchanged	Unchanged

## RESULTS

*The contraction of the Biebl loop*

It was possible to distinguish two types of activity in this preparation, one characterized by regular rhythmic contractions, and the other by irregular strong slow contractions. The frequency of these rhythmic contractions was remarkably constant for an individual animal and varied very little from day to day. This is shown in Table II. The strong slow contractions were most frequently observed during the six hours following feeding. They became greatly reduced in magnitude and frequency after food was withheld for more than 24 hours. These different types of movement are shown in Fig. 1.

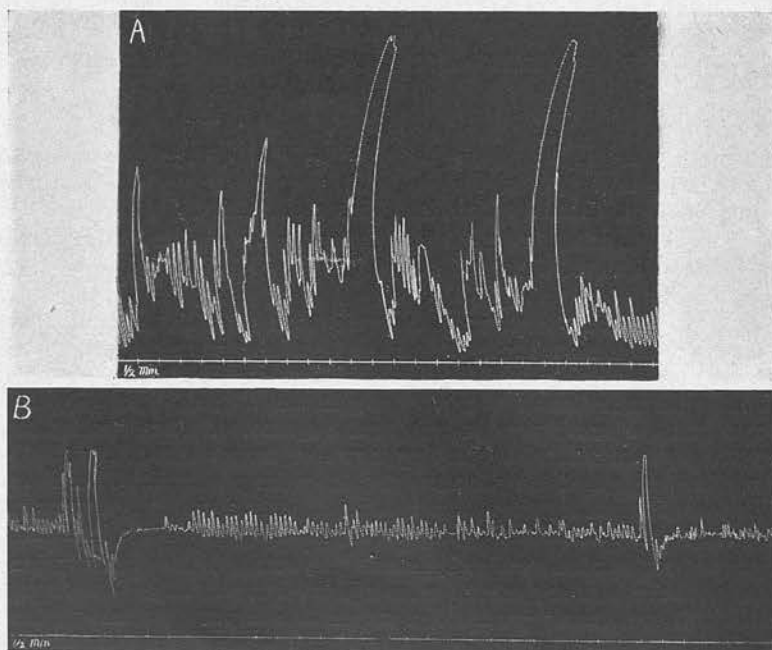


FIG. 1.—(A) Motility in a Biebl loop one hour after feeding; the large slow contractions predominate. (B) The same animal 24 hours after feeding; the large slow contractions occur infrequently.

TABLE II  
THE RHYTHMIC CONTRACTIONS OF THE HORSE ILEUM

	Biebl loop			Thiry-Vella loop
	Pony I	Pony II	Pony III	
Mean rate .. ..	10.23±0.49/min.	12.6±0.95/min.	10.5±0.51/min.	8.1±0.69/min.
Range .. ..	9 to 11/min.	10 to 15/min.	10 to 11/min.	7 to 9/min.
Period of observation	3 months	3 months	2 months	2 months
Number of observations	65	102	30	32

*The contractions of the Thiry-Vella loop*

The contractions shown by this preparation were similar to the rhythmic contractions of the Biebl loop. Their regularity is shown in Table II. Fasting for periods of 24 and 48 hours did not change the type or frequency of contraction. When isotonic solutions of sodium chloride and sodium bicarbonate, sodium dihydrogen phosphate and disodium phosphate were introduced into the oral opening they were forcibly ejected from the other opening, whereas the recording balloon was not propelled through the loop. Changes in tone were not conspicuous.

*The effect of drugs on ileal motility*

*Adrenaline.*—Adrenaline inhibited motility in both Biebl and Thiry-Vella loops (Fig. 2). This agreed with observations on the perfused ileum. The characteristic sweating produced by this drug on the horse was always observed.

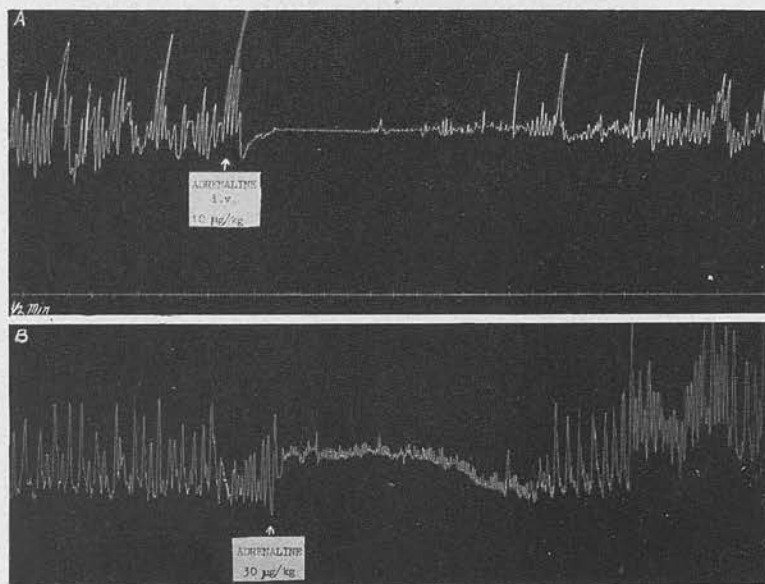


FIG. 2.—The effect of adrenaline on the movements of (A) a Biebl and (B) a Thiry-Vella loop.

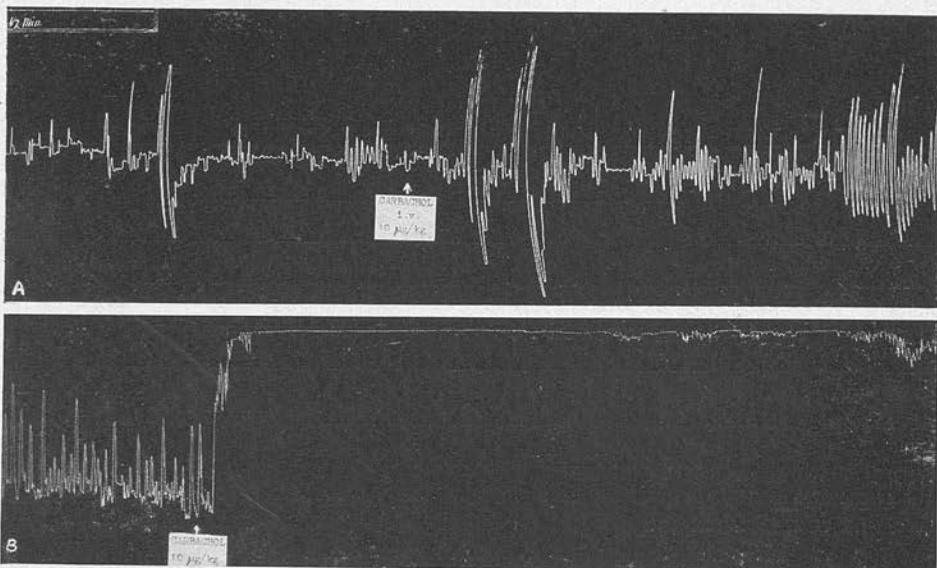


FIG. 3.—The effect of carbamylcholine on the motility of (A) a Biebl and (B) a Thiry-Vella loop.

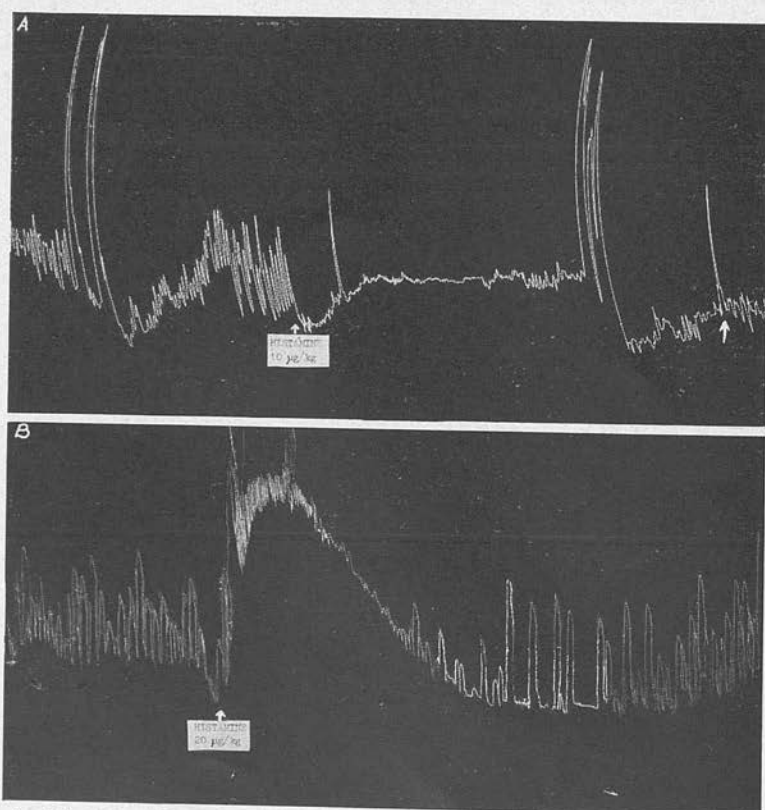


FIG. 4.—The effect of histamine on the motility of (A) a Biebl and (B) a Thiry-Vella loop.



*Carbamylcholine.*—In two ponies out of three with a Biebl loop carbamylcholine increased motility (Fig. 3). The third pony showed at different times increased and decreased motility. This different response can be explained by the recording technique, which did not distinguish between a long sustained contraction and inhibition. Such an explanation was supported by the experiments on the Thiry-Vella loop, which responded to carbamylcholine with a long sustained contraction. Hence the effect of this drug on the ileum of the living horse was similar to that on isolated preparations.

*Histamine.*—This drug appeared either to inhibit movements of the Biebl loop or to produce a long sustained contraction. However, as the Thiry-Vella loop

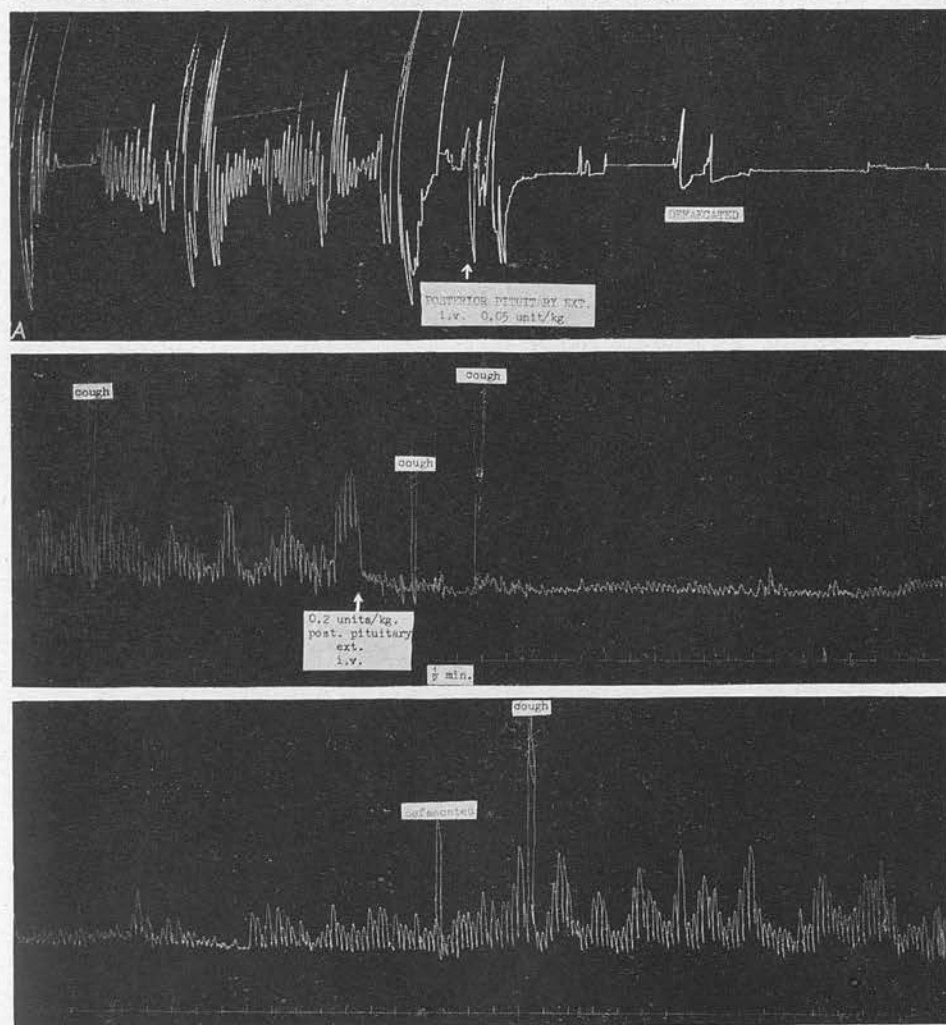


FIG. 5.—The effect of posterior pituitary extract on (top) a Biebl loop and (middle and bottom) a Thiry-Vella loop.



showed a marked rise in tone it is probable that the lack of movement shown by the Biebl loop (Fig. 4) was due to a sustained contraction. The response of the ileum of the intact animal, therefore, appeared similar to that of the isolated ileum (Alexander, 1949).

Immediately after the intravenous injection of histamine the animal showed a greatly increased respiratory rate, defaecation, and an increased secretion of tears and nasal mucus. The electrocardiograph showed an increased heart rate.

*Posterior pituitary extract.*—The injection of posterior pituitary extract was followed by inhibition in Biebl and Thiry-Vella loops (Fig. 5). This was unexpected in view of previous findings on the isolated strip and perfused ileum, when posterior pituitary extract had either no effect or caused a slight contraction (Alexander, 1949).

The recording balloon was not propelled through the Thiry-Vella loop by carbamylcholine, histamine, or posterior pituitary extract.

Various explanations of this disparity were considered. Earlier work (Alexander, 1949, 1950) had shown that the main factor influencing motility in the perfused ileum was the supply of oxygen. The possibility was therefore considered of the inhibitory effect of posterior pituitary extract being produced indirectly by the action of this drug on intestinal blood flow.

#### *The effect of drugs on the temperature of the ileum*

By observing the temperature of an exposed structure it is possible to infer whether the rate of blood flow has changed. This method, on account of its convenience, was used to study variations in blood flow through the Biebl and Thiry-Vella loop after the intravenous injection of the four humoral agents. The results of these experiments are shown in Fig. 6. It was clear that the inhibition of motility

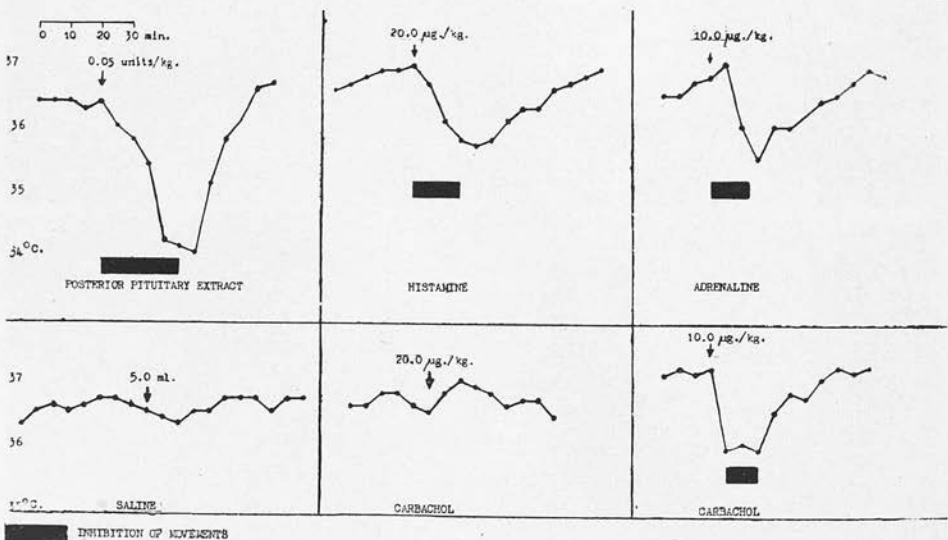


FIG. 6.—The effect of posterior pituitary extract, adrenaline, histamine, and carbamylcholine on the temperature of the intestine of the living horse.

after the intravenous injection of posterior pituitary extract was always accompanied by a profound fall in ileal temperature, and, in the Thiry-Vella loop, pallor followed by cyanosis of the exposed "cuff" of mucous membrane; simultaneously there was a marked slowing of the heart rate.

A fall in temperature always occurred after the injection of adrenaline, but the inhibition of motility preceded the fall by at least five minutes. This observation supported the agreement between the inhibitions produced by this drug *in vitro* and *in vivo*, thus showing adrenaline to have a direct effect on the ileum.

The irregularity of the response in the intestinal temperature after the intravenous injection of carbamylcholine was particularly interesting. The two ponies with Biebl loops which responded to carbamylcholine with increased motility did not show any fall in intestinal temperature. The third pony, which occasionally displayed an apparent inhibition of movement, showed, on these occasions, a fall in intestinal temperature.

In all instances where temperature of the mucosal "cuff" of the Thiry-Vella loop fell, the mucous membrane became cyanotic.

#### DISCUSSION

*Normal motility.*—The movements recorded from the Biebl loop in the horse were similar to those obtained from the Biebl loop in the dog (Castleton, 1934; Douglas and Mann, 1939). The records from the Thiry-Vella loop, however, showed only regular rhythmic contractions, but not the propulsive contractions exhibited by similar preparations in the dog (Krueger, Lampe, and Reid, 1936). Further, there seemed no tendency for the balloon to traverse the loop; this agreed with the observations on the isolated perfused ileum, where only about one loop in four showed rapid propulsion (Alexander, 1950). These findings suggest that the large slow contractions shown by the Biebl loop might be associated with the propulsion of digesta through the ileum.

*The effect of drugs.*—The effect of adrenaline on the ileum was essentially the same in the living animal as on isolated tissues (Alexander, 1949). The inhibitory effect of this drug preceded by at least five minutes any reduction of intestinal temperature, and showed that its effect was produced directly on the ileum.

Carbamylcholine usually increased the motility of Biebl loops, and the marked rise in tone produced by this substance would account for the apparent inhibition which was occasionally recorded. It was, however, of some note that on the occasions where an inhibitory effect was recorded there was an associated fall in intestinal temperature.

The effects of histamine on the preparations of horse ileum *in vivo* and *in vitro* were not difficult to reconcile. The apparent inhibition produced by this drug on the Biebl loop could be due to the associated increase in tone. However, this drug always caused a fall in intestinal temperature which accompanied the inhibitory effect recorded from the Biebl loop.

The most interesting observation was, however, the disparity between the inhibition produced by posterior pituitary extract on the ileum of the living horse and the lack of effect of this drug on the isolated ileum. Since the inhibition was shown by both preparations of the ileum in the living animal it could not be due to any artifact in the recording technique. Earlier work (Alexander, 1950) had shown that

the main factor influencing motility in the perfused ileum is the supply of oxygen. Moreover, it has long been known that posterior pituitary extract decreases the portal blood flow in cats (Clark, 1928). The intestinal temperature in both Biebl and Thiry-Vella loops showed a profound fall after the injection of this posterior pituitary extract, and, in the Thiry-Vella fistula, cyanosis of the mucous membrane. It seemed clear, therefore, that the intestinal blood flow had been greatly diminished, and this, in view of the earlier work (Alexander, 1950), would account for the decreased motility. The effect of posterior pituitary extracts on the alimentary tract has been reviewed recently by Stehle (1950), who states that, "In the intact animal the intense constriction of the splanchnic blood vessels caused by the pressor hormone makes one think of the possibility of an indirect action." The experiments on the horse support this idea.

The occasional inhibition which followed the injection of carbamylcholine was always associated with a fall in intestinal temperature, suggesting that even the well-marked intestinal stimulation usually produced by this drug could be masked by its action on the circulation. Further, with a well-recognized inhibitor of intestinal movement such as adrenaline, the ileal inhibition preceded the fall in temperature by at least five minutes, showing that the inhibition was direct and not secondary to a reduced blood flow.

The concurrence of reduced motility, a fall in intestinal temperature, and cyanosis of the ileal mucosa lends strong support to the evidence from the perfused ileum (Alexander, 1950) that, in the horse, ileal motility is greatly influenced by any interference with its supply of oxygen.

#### SUMMARY

1. Ileal motility has been studied in three ponies with Biebl loops and one with a Thiry-Vella loop.
2. The movements of the exteriorized ileum in the recently fed pony with a Biebl loop comprised a mixture of strong slow contractions and quicker rhythmic contractions. In the fasting animal the rhythmic contractions predominated. The Thiry-Vella fistula showed only rhythmic contractions.
3. Adrenaline inhibited, and carbamylcholine usually increased, ileal motility in the living animal.
4. Posterior pituitary extract diminished contractions in both Biebl and Thiry-Vella loops and caused a prolonged fall in ileal temperature.
5. A fall in ileal temperature was always associated with inhibition of motility.
6. It is suggested that intestinal motility in the horse is greatly influenced by drugs which affect intestinal blood flow.

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The available evidence shows that the blood sugar concentration in ruminants is much lower than in horses (Boyd, 1950). Since, in the ruminant, soluble carbohydrates are rapidly fermented

(Phillipson and Mainally, 1942), it has been suggested that the low blood sugar level in these animals is due to their reliance on the conversion

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(Boyd, 1950). Fermentative digestion in the horse

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The available evidence shows that the blood sugar concentration in ruminants is much lower than in horses (Reid, 1950). Since, in the ruminant, soluble carbohydrates are rapidly fermented (Phillipson and McAnally, 1942), it has been suggested that the low blood sugar level in these animals is due to their reliance on the conversion of propionic acid as the main source of glucose (Reid, 1950). Fermentative digestion in the horse is carried out in the large intestine. Hence, digesta must first undergo the digestive processes of the stomach and small intestine. It might be argued, therefore, that the opportunity exists in the horse for the digestion and absorption of soluble carbohydrates before the digesta is fermented and this might account for the higher blood sugar level in this species. However, comparatively few studies of the blood sugar concentration in the horse have been made and the range of these is considerable, Holt and Reynolds (1924) giving a range of 78 - 370mg./100 ml. blood. So far as can be ascertained no information is available about the digestive and absorptive functions of the horse stomach and

small intestine. The object of the experiments described here was to determine the variation in blood sugar concentration in individual horses over a period of several months and to determine whether appreciable amounts of a soluble sugar, such as glucose, were absorbed from the ileum.

### Methods

The concentration of glucose in blood and intestinal fluids was determined by the method of Somogyi (1945, 1952). Blood was taken from the jugular vein into dry bottles containing, as anticoagulant, sodium fluoride. Ileal contents were obtained by introducing a wide-bore hypodermic needle into an exteriorized loop of ileum, prepared in the manner described previously (Alexander, 1951) and caecal contents from a pony fitted with a permanent cannula in the caecum (Alexander, 1952). Sodium fluoride was omitted from samples intended for the yeast fermentation experiments. In these experiments, a little well-washed brewer's yeast was added to the blood or other fluid and the sample incubated overnight at 37°C. Tubes containing a pure solution of glucose with and without yeast were treated in the same fashion. Total volatile fatty acids were



estimated in the manner already described

(Alexander, 1952). In the experiments in which glucose was administered, this substance was given by stomach tube in a 50% (W/V) solution except for a few experiments in which solid glucose was fed, the animals used in these experiments were ponies weighing between 150 and 230 kgs., trained to stand quietly whilst blood and other fluids were removed.

### Results

Estimations of the blood sugar concentration in eight ponies gave values within the range found by Stewart and Holman (1940). The results of these experiments are shown in Table 1. It was clear from these results that the normal concentration of sugar in horse blood was appreciably higher than in ruminant blood, for example, Reid(1950) found that non-pregnant sheep had a mean value of  $39.1 \pm 3.4$ mg. glucose/100 ml. blood. Since no information could be obtained about the fate of soluble carbohydrates when fed to horses, experiments were carried out to determine whether orally administered glucose produced a hyperglycaemia.

### Effect of orally administered glucose

Glucose, when given to ponies by mouth,

produced a rise in blood sugar concentration which returned to the pre-administration level within four hours (Table 11). It appeared from these results that the glucose rapidly reached the site of absorption. This appeared to be the small intestine. Experiments on a pony with an exteriorised loop of ileum made possible the simultaneous sampling of venous blood and ileal contents. The pony was given glucose by stomach tube and, at intervals afterwards, samples were taken of blood and ileal contents. The results of a typical experiment are shown in Fig. 1. Similar results were obtained in four such experiments. These experiments showed that the hyperglycaemia produced by feeding glucose was associated with a marked increase in the glucose concentration of the ileal contents.

Since digesta passes along the horse ileum within three hours, experiments were carried out to determine whether orally administered glucose escaped absorption in the ileum. A pony with a caecal fistula was used for these experiments. The glucose concentration of blood and caecal fluid was estimated at intervals following the oral administration of glucose. The results of a typical experiment are shown in Fig. 2. This showed no appreciable amount of glucose to have accumulated in the caecum. However, it did not

exclude the possibility of glucose reaching the caecum and being fermented so quickly that none accumulated. Since caecal contents ferment glucose to produce fatty acids (unpublished observations) estimations of the volatile fatty acid concentration in caecal contents were made at intervals after giving glucose; there was no increase in volatile fatty acid concentration. It seemed clear from these experiments that orally administered glucose was absorbed from the small intestine.

Since foods such as hay contain more than 4% of their dry weight as reducing substances, it was of interest to determine the amount of reducing substances normally present in the ileum of a pony on a diet of hay. It was found that ileal juice from a hay fed pony contained a substance which was not precipitated by zinc hydroxide, reduced Somogyi's reagent and was fermented by brewer's yeast. This substance occurred in quantities up to the equivalent of 97 mg. glucose per 100 ml. ileal juice and its appearance was related to feeding. A typical experiment is shown in Table III.

#### Effect of Withholding Food

In these experiments the food was withheld for a period of 72 hours, the animal having free access to water at all times. In three out of four ponies this produced a fall in blood sugar

concentration at the end of the first 48 hours of fasting, thereafter, the concentration rose to the pre-fasting level although no food was given for a further 24 hours. This is shown in Fig. 3.

#### Clearance of Injected Glucose.

Since the hyperglycaemia produced by the oral<sup>administration</sup> of glucose lasted for less than four hours, the horse seemed to be able to metabolise glucose readily. It was found that intravenously injected glucose (0.25 gm./kg.) disappeared from the blood in less than  $2\frac{1}{2}$  hours. This experiment was carried out on a group of five ponies with similar results in each case.

#### Discussion

In a survey of 141 horses, Holt and Reynolds (1924) found the range for blood sugar between 78 - 370 mg./100 ml. and in a similar study Stewart and Holman (1940) gave a range of 54 - 95mg./100 ml. However, both these studies were concerned with single observations on a large number of animals and gave no information about the variation within a single animal. The experiments described here show the fluctuation in the blood sugar concentration in individual animals over periods of up to three years. Most

of the values obtained in the eight animals under observation were within the range of Stewart and Holman's (1940) results and it is difficult to accept those given by Holt and Reynolds(1924) as applying to normal horses. It is well known that excitement produces a rapid rise in blood sugar and as the figures obtained by Holt and Reynolds were single observations, it is possible that some of their very high values were due to some such complication. The normal blood sugar concentration in the eight ponies studied was almost twice as high as in ruminants (Reid,1950). The reason for this difference was probably due to the ruminant rapidly fermenting carbohydrates in the rumen (Phillipson and McAnally, 1942). In fact, Heald(1951) has shown that only 5 - 6 g. of glucose leave the abomasum of a sheep in 24 hours. The experiments described here showed that glucose was absorbed from the small intestine in the horse and produced a hyperglycaemia. This was in contrast to the ruminant ( Bell and Jones, 1945). The higher blood sugar concentration of the horse might be due, therefore, to the soluble carbohydrates of the diet being absorbed from the ileum. These substances are by no means inconsiderable. Ferguson(1946) showed that in grass 5% of the dry matter consisted of

reducing substances. This explanation was supported by the finding of appreciable concentrations of a glucose-like substance in the ileal contents of the pony recently fed hay.

The time of 1 - 2 hours after feeding glucose from the maximum rise in blood sugar concentration was in agreement with the time the ingested material might be expected to reach the ileum (Alexander and Benzie, 1951). In the experiment illustrated, the rise in glucose concentration of the blood preceded the maximum concentration in the ileal contents. This was probably due to the exteriorised loop being made in the mid-ileal region. Therefore, time was allowed for a quantity of the glucose to be absorbed in the jejunum before appreciable amounts reached the loop. Further, absorption from the ileum must be a fast process since the rapid transit of digesta through the ileum (Alexander and Benzie, 1951) did not prevent the complete absorption of the relatively large quantities of glucose given. This was shown by the failure to detect appreciable amounts of glucose in the caecal liquor after feeding large quantities or to find a rise in volatile fatty acid concentration.

In a study of the clearance of intravenously injected glucose in horses, Link (1940) found that



the blood sugar concentration had not returned to the pre-injection level in 24 hours. This observation could not be confirmed and in similar experiments it was found that the blood sugar concentration returned to the pre-injection level in less than 24 hours. However, Link's experiments in which 50 g. of glucose given intravenously, failed to cause glycosurea showed the horse capable of metabolising large quantities of this sugar.

The response to fasting of the horses' blood sugar concentration differs from that found in sheep. Fasting the ponies usually caused a fall in blood sugar concentration followed, after 48 hours, by a rise. This response resembled that found in man, pig, cat, guinea-pig (Shope, 1927) and cow (Robertson and Thin, 1953). In non-pregnant sheep, fasting for 48 hours had no effect on the blood sugar level (Reid, 1950, Frazer, Snook Godden and Thompson, 1939). It appeared, therefore, that the horse was able to digest and absorb soluble carbohydrates without these being fermented. The horse, therefore, appears to be, in this respect, better adapted to the herbivorous mode of life than the ruminant which ferments indiscriminately all ingested food.

Summary.

1. The blood sugar concentration has been studied in 8 ponies over a period of from 3 - 36 months.
2. Orally administered glucose caused a hyperglycaemia associated with the presence of a large concentration of glucose in the ileum.
3. Within three hours of feeding hay a yeast fermentable reducing substance appeared in the ileal contents in concentrations equivalent to 40 - 90 mg. glucose/100 ml. fluid.
4. Withholding food usually caused a fall in blood sugar concentration during the first 48 hours; thereafter, it rose to the normal level.
5. It is suggested that the horse, in contrast to the ruminant, is able to digest and absorb soluble carbohydrates without subjecting them to fermentation.

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TABLE 1.

Concentration of Glucose in the Blood  
of Normal Ponies.

Pony	Sex	Number of Observations	Period of Observations	Mean Blood Sugar mg/100ml
Harriet	F.	36	18 months	70.4 <u>+11.0SD</u>
Rufus	G.	10	4 "	70.6 <u>+10.5</u>
Tilly	F.	28	18 "	78.9 <u>+18.9</u>
Blackie	F.	13	12 "	66.3 <u>+ 8.1</u>
Ginger	F.	14	3 "	79.1 <u>+12.9</u>
Judy	F.	7	8 "	88.0 <u>+12.5</u>
Ginger F.	G.	33	36 "	<del>100.1</del> <u>+24.6</u>
Black F.	F.	31	36 "	66.0 <u>+17.1</u>

**TABLE II**

**The Effect on Blood Sugar Concentration of Orally Administered Glucose**

Pony	Maximum Increase		Return to pre-feed level		Dose
	Time after Feeding	Concentration	Time after Feeding	Concentration	
Harriet	3.5 hours 2.0 hours	151.2mg./100ml. 89.7	- 2.5 hours	- 62.2 mg./100 ml.	1 gm./kg. 0.5
Rufus	1.5 1.0	100.2 112.5	3.5 3.5	73.5 68.2	0.5 given as 0.5 solution
Ginger F.	1.0 1.5	154.0	4.0 4.0	91.0 77.0	0.5 0.5 ..
Black F.	1.0	100.0	4.0	62.0	0.5
	1.0	84.2	4.0	60.5	0.5 ..
	1.0	89.0	4.0	79.0	0.5
	1.5	106.0	4.0	89.0	1.0
Judy	2.0	147.0	4.0	84.5	1.0
	1.0	135.0	3.0	78.0	1.0 ..
	1.0	118.0	3.0	93.0	1.0
	2.0	128.0	4.0	74.0	1.0

Table 111

Concentration of Reducing Substance in Ileal

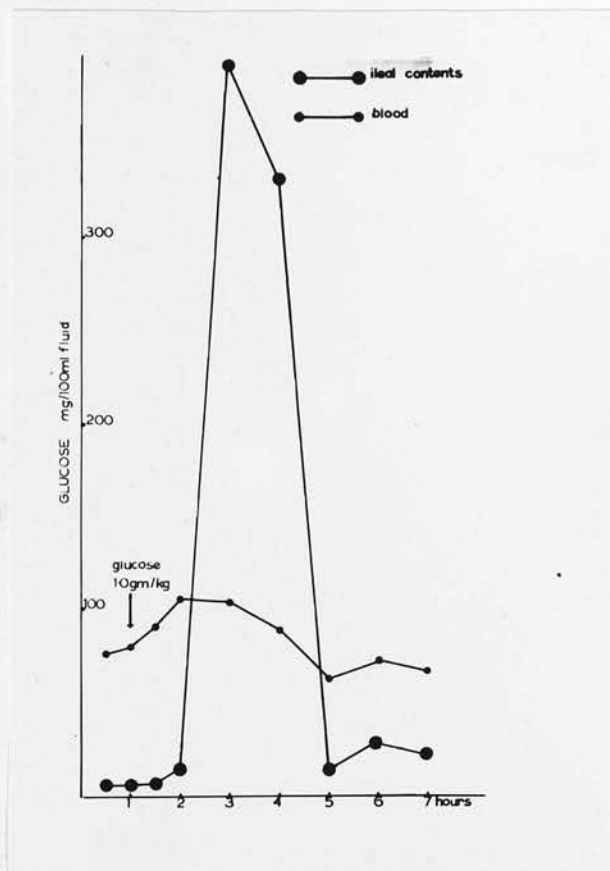
Liquor of a Hay-fed Pony.

Time	Reducing substance as glucose	Remarks
9.30 a.m.	0.0 mg./100 ml. liquor	Fed Hay
12.00	40.0    "    "	
3.00 p.m.	16.0    "    "	

The effect of feeding glucose on the  
concentration in blood and ileal contents.



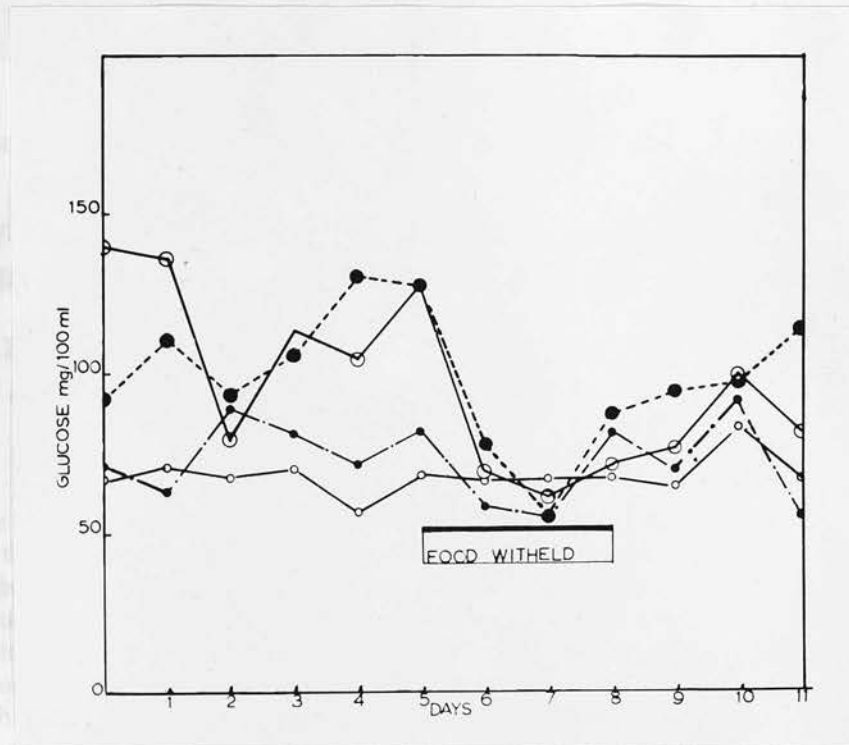
Fig.1



The effect of feeding glucose on the concentration in blood and ileal contents.

The effect of feeding glucose on the glucose concentration in blood and ileal contents.

Fig.3



The effect on the blood sugar concentration of withholding food from 4 ponies.

## A REVIEW OF KNOWLEDGE AVAILABLE CONCERNING DIGESTION IN DOMESTIC HERBIVORA

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### PART I

THE basic difference between herbivorous and omnivorous or carnivorous animals lies in the ability of herbivores to digest cellulose and related substances. Since mammals do not secrete cellulases, they depend on the utilization of the products of microbial activity. This has made it necessary to modify the digestive tract to provide a suitable environment for microbial fermentation. The usual adaptation has involved the enlargement of part of the tract to form a fermentation chamber. Most commonly the parts to undergo this modification have been the cæcum and colon. However, some species have in addition an enlarged part of the stomach. A good example of the first type of development is shown in the Equidæ and of the last in the Ruminant (Table I).

In their review of Ruminant Digestion, Elsdon and Phillipson (1948) state that ruminants are anatomically better adapted to the herbivorous mode of life because they subject their ingesta to two fermentations; one before and the other following the usual mammalian digestive processes. Whether such a double fermentation is advantageous is open to question since ruminants ferment indiscriminately in the reticulo-rumen water-soluble carbohydrates (McAnally and Phillipson, 1942) as well as cellulose; they also deaminate amino-acids (Shazly, 1952a) producing ammonia, CO<sub>2</sub> and volatile fatty acid. Whereas the horse, for example, absorbs water soluble carbohydrates such as glucose directly from the small intestine (Alexander, 1954). Moreover, Bonnazzi (1951) found the horse digested more protein than either the cow or pig, probably because of the digestion of proteins in the small intestine without subjection to fermentation. However, both ruminant and monogastric herbivora under natural conditions have a similar diet and depend on the products of fermentation for much of their absorbable nutriment. Since the substrate for fermentation must differ in these types of herbivora, in the ruminant all digesta being liable to fermentation in the rumen whereas in the non-ruminant some parts of the diet are absorbed and the residue fermented, a study of digestion in two such species would probably help the understanding of the fundamental mechanisms of herbivorous digestion. The horse and domestic ruminant provide good examples of colon fermenting and stomach fermenting herbivores, respectively. Moreover, these species are very liable to diseases of digestion and are therefore of particular interest to veterinarians. The purpose of this review is to compare as far as possible digestion in the horse and ruminant and, in particular, to indicate the absence of information.

TABLE I

Relative capacity of the parts of the digestive tract (per cent)				Ratio of capacity of total digestive tract (Litres)/body-weight (kg.)
Stomach		Small Intestine	Large Intestine	
Cat ... ..	70	15	15	16
Pig ... ..	30	33	37	25
Horse ... ..	9	30	61	33
Ox ... ..	71	19	10	50

Calculated by G. A. Levvy from data of Colin, G. (1886). *Trait. Physiol. Comparée*.

### Mechanical Factors

The motor activities of the alimentary tract are fundamental to the digestive processes, serving to mix the digesta with the digestive secretions, to expose this mixture to the epithelium of the tract and to propel the residue along the bowel.

The movements of the reticulo-rumen have attracted the most interest and present many special features. They show a basic sequence of events which is subject to considerable variation and has been carefully analysed by Phillipson (1939). The reticulum shows a regular double contraction occurring about once each minute; this contraction forces the liquid digesta into the rumen. The reticulum then relaxes and fills with digesta from the rumen. The contractions of the rumen are slower and less forceful than those of the reticulum, the rumen contracting once or twice for each double reticulum contraction; the dorsal and ventral sacs of the rumen usually contracting alternately. This has been confirmed recently by Brunard and Dussardier (1953a). These authors have also directed attention to the vestibule of the rumen which appears to be a specialised part of this organ. The vestibule, prepyloric part of the abomasum and the reticulum are the only parts of the ruminant stomach to show spontaneous motility in freshly killed animals.

Stigler (1931, 1933) who has contributed to the study of rumination over the past twenty years has recently (1949) described a working model to illustrate the rumination mechanisation. This would probably be of value in teaching institutes.

Wise, Link, Thompson and Caldwell (1946) rumenectomised calves and found them to suffer no ill-effects other than a tendency to bloat and unthriftness which was alleviated by the administration of rumen juice. The rumen tended to regenerate. Piana (1952) has made observations on rumenectomised sheep. He found digesta to pass much more quickly along the alimentary tract than it did in normal sheep. It has long been known that the rumen developed in size as the young ruminant changed from a diet of milk to a normal herbivorous diet. Additional evidence of the effect of diet on rumen development has been provided by Blaxter, Brown and Macdonald (1952), who found that calves fed roughage had an increased capacity of their fore-stomachs, their large bowel was unchanged in capacity. An analogous change occurs in the horse (Alexander and Benzie, 1951). In weaned foals the cæcum and ventral colon

occupied a much greater proportion of the abdominal cavity than in suckling foals. This development is presumably to provide the fermentation chamber necessary for the microbial digestion of the herbivorous diet.

The embryology of the bovine stomach has been studied by Becker, Arnold and Marshall (1951). These authors found the rumen to be the largest chamber of the stomach in early foetal life, whilst at term the abomasum accounted for half the total weight of all parts of the stomach. Differentiation of the honeycomb occurred between 72 and 100 days. Duncan and Phillipson (1951) studied the motor activity of the stomach in foetal sheep. They found the non-functional period to end after 50-60 days of gestation, but sustained activity was not seen until the 70th day; foetal swallowing began soon after this time.

Some of the factors influencing the movements of the bovine stomach have been studied by Balch (1952). He found the reticulum contractions were quickest when the animal was eating and slowest during rumination. Omasal activity has been investigated by Balch, Kelly and Hein (1951) who concluded that digesta passed continuously from reticulum to omasum probably with each reticular contraction. The records shown by this group of investigators resemble the earlier records such as those of Schalk and Amadon (1928) who found continuous activity in the omasum consisting of slow powerful contractions. Brunard and Dussardier (1953*b*) have published tracings showing a relationship between reticulum contractions and the contractions of the omasum. They distinguished two types of omasal contractions. The abomasal contractions described in this same paper are remarkably similar both in form and frequency to the contractions of the horse stomach (Alexander, 1951*a*).

Phillipson (1952) has studied the flow of digesta from the duodenum and found that about 300-450 ml. passed from the abomasum each hour. Distension of the duodenum decreased abomasal emptying as did the re-introduction of abomasal contents into the duodenum. More digesta left the abomasum after feeding cereal offals and meals than after feeding hay. There appeared to be no correlation between reticulum motility and abomasal emptying. Singleton (1951) has described abomasal-duodenal inhibitory reflexes in the goat similar to those of the dog; olive oil in the duodenum consistently produced abomasal inhibition whereas protein breakdown products only inhibited contractions on half the occasions tried.

The movements of the horse's stomach have received very little attention since the early studies of Colin (1886) who experimented on the stomachs of freshly killed horses. Colin found that when the pylorus was ligatured and intragastric pressure increased, the stomach ruptured before any contents escaped through the cardiac sphincter. The rupturing of the equine stomach has been studied in greater detail by Neumann-Kleinparl and Schultze (1940). They found that rupturing took place parallel to the greater curvature and the pressure required was between 55 and 69 gm./sq. cm. The normal intra-gastric pressure was 10 gm./sq. cm. and in tympany it rose to 50 gm./sq. cm. Schalk and Amadon (1928) recorded intra-gastric pressures in the living horse, and found that powerful contractions occurred during feeding; these contractions did not

recur until 5 hours after feed. Schalk and Amadon termed these latter contractions "hunger contractions." However, it is very doubtful whether true hunger contractions occur in the horse (Alexander, 1951*a*). Observations on gastric contractions in the horse have been published recently by Brunard and Dussardier (1952*a*). The observations were made on animals anaesthetised with chloralose and the records obtained from a balloon passed into the stomach through an opening in the oesophagus. These experimental conditions may account for the difference in frequency of the stomach contractions recorded by Brunard and Dussardier (1952*a*) and those described by Alexander (1951*a*). The latter author found that only when the tube in the oesophagus was less than 6.5 mm. diam. was it possible to obtain a record of normal gastric movements.

The functions of the ileum have received little attention although the importance of this part of the digestive tract in ruminants is shown by the experiment of Besselaar and Quin (1935). These workers extirpated the lower half of the ileum in sheep and found that death took place within 12 months and was preceded by cachexia and diarrhoea; exclusion of the caecum and upper colon had a similar effect. There was also a disturbance of water metabolism.

The paucity of information on the functions of the intestine of the horse has been due in the past to the difficulty of preparing permanent fistulae into the various parts of the alimentary tract. However, it is now possible to make fistulae into both small and large intestines (Alexander, 1951*b*, 1952). From *in vitro* and *in vivo* studies of the horse ileum it appears that any diminution in the oxygen supply to this organ, is accompanied by an inhibition of movement, (Alexander, 1949, 1950, 1952). It would be interesting to know whether the ruminant ileum is similarly affected by anoxia.

Kuznetsova (1950) connected the duodenum of a foal to the jejunum by means of rubber tubing. He found that 90-140 litres of chyme passed each day. This was a much greater volume than could be accounted for by ingested food and water. It should be noted with regard to this large volume of chyme that Colin (1886) found that a small horse eating hay secreted 5 kg. of saliva in an hour.

Although the caecum and colon are the most important sites of fermentative digestion in the horse, even in ruminants these organs are not unimportant, about 30 per cent of the digestible cellulose in the diet being digested in the caecum and colon (Gray, 1947). However, the mechanics of the ruminant large intestine have attracted little attention. Sporri and Ascher (1940) studied the movements of the caecum and colon by introducing barium through a fistula in the caecum of goats and making radiological observations. They observed mixing movements, peristalsis and anti-peristalsis in the caecum and proximal colon, and peristalsis and segmentation in the spiral colon. Pellet formation was first noted in the caudal part of the spiral colon. The backward passage of barium through the ileo-caecal opening into the ileum was also observed.

One of the main reasons for the lack of information on the functions of the large intestine of the horse has been the surgical problem presented in preparing permanent fistulae into this organ. However, Alexander and Donald



(1948) have described a method of fistulating the cæcum and large colon in ponies and this method has now been developed (Alexander, 1952) so that fistulae can be produced in the cæcum, dorsal and ventral colons of the same horse. These techniques should greatly facilitate the study of digestion in the horse. Howell and Cupps (1950) have described experiments on a horse with a permanent fistula of the cæcum; they had no device for closing the fistula. These authors found that feeding increased cæcal movements. Alexander (1952) using unanæsthetised fistulated ponies has described three types of cæcal and colonic movement. He found withholding food greatly decreased motility in the cæcum and ventral colon but had little effect on the dorsal colon; there was no evidence of increased activity on feeding. Brunard and Dussardier (1952*b*) have recorded the movements of the cæcum in anæsthetised (chloralose) equines. They have described strong emptying contractions of this organ. However, their published tracings show a much slower rate of contraction than in tracings published by Howell and Cupps (1950) or Alexander (1952). Indeed, the tracings of Brunard and Dussardier resemble those from a fasting horse (Alexander, 1952). An explanation for this discrepancy might lie in some inhibitory mechanism operating in anæsthesia.

#### Innervation

The trend of evidence during the past twenty years has been towards the localisation of an area in the brain which controls the movements of the reticulo-rumen. Popov, Kudryavcen and Krasowsky (1933) showed the movements of the ruminant stomach to be dependent on an intact vagal supply. These workers exteriorised the vagi in the neck and observed that cessation of stomach movements was produced by cooling these exteriorised nerves. However, stimulation of the central end of the vagus nerve failed to cause rumination (Aggazzotti, 1910). Dukes and Sampson (1939) found stimulation of the peripheral vagus caused slow contractions of the omasum and characteristic contractions of the abomasum. Brunard and Dussardier (1951) studying the effect of vagal stimulation on gastric motility in chloralosed ruminants, found that stimulation caused contraction of the stomach, slowed the heart rate and lowered blood pressure. However, when acetylcholine, the accepted mediator of vagal stimulation, was injected intravenously it caused inhibition of gastric motility. The inhibitory effect of acetylcholine had been described earlier by Quin and van der Wath (1938). However, the related choline ester, carbamylcholine, and the choline esterase inhibitor, physostigmine, were found by Clark (1950) to increase ruminal activity. The reason for this conflict of evidence between the increased activity resulting from vagal stimulation and the inhibitory effect of acetylcholine is not clear. Brunard, Dussardier and Labouche (1950) found that acetylcholine caused contraction of strips of rumen and omasal muscle *in vitro* and Brunard and Dussardier (1951) showed that atropine inhibited the effect of vagal stimulation. This evidence they interpret as showing that a choline ester other than acetylcholine mediates the effect of vagal stimulation in the ruminant. However, since their evidence shows that the effect of

vagal stimulation is inhibited by atropine and that it resembles the direct action of acetylcholine on the stomach muscle, the most probable explanation of the inhibition produced by the injection of acetylcholine is that this is an indirect effect. Such an indirect effect might be caused by an interference with the blood flow to the stomachs or to a stimulation of the adrenal medulla causing an excessive secretion of adrenaline. It has been shown in the case of the horse that the effect of carbaminoylcholine and posterior pituitary extract on the movements of the ileum can be masked by their effects on circulation (Alexander, 1952).

Comline and Titchen (1951) found that splanchnic stimulation in decerebrate, spinal or chloralosed goats caused a small reticulum contraction and the intravenous injection of adrenaline had the same effect. Brunard and Dussardier (1951) found adrenaline and nor-adrenaline to act in a similar way although clear evidence has been obtained of the inhibition of reticulo-ruminal motivity by adrenaline in the unæsthetised animal (Duncan, 1951). Moreover, work on the horse has shown that adrenaline can cause either contraction or inhibition of ilial muscle depending on the oxygenation of the tissue (Alexander, 1949).

The earlier work on the innervation of the ruminant stomach has been described by Hoflund (1940) who himself worked extensively in this field. However, work during the past decade has repeated and extended these early observations. It is clear that the normal propulsive activity of the reticulo-rumen is dependent upon at least half of the vagal nerve supply remaining intact. Rumination and the œsophageal groove reflex are similarly dependent upon the vagus. Vagal section produces delayed emptying and dilation of the abomasum. The absence of movement in the reticulo-rumen following vagal section is unaffected by splanchnectomy (Duncan, 1953). Comline and Titchen recently (1951) made a detailed study of the œsophageal groove reflex using decerebrate calves and lambs. They found that the superior laryngeal nerve was the only nerve of which stimulation consistently caused reflex contraction of the groove. The entry of water into the posterior part of the mouth was a more certain stimulus than nerve stimulation. The reflex was inhibited by stimulation of the central end of the abomasal nerve, this inhibition being independent of splanchnic innervation. They were unable to find any evidence of an inhibitory sympathetic innervation of the groove. Atropine abolished contraction of the groove resulting from reflex excitation or stimulation of the dorsal vagus. Reflex excitation was abolished by the intravenous injection of adrenaline and nor-adrenaline.

Duncan (1953) suggests that the reticulo-rumen, which represents the fundus of the primitive stomach, has developed an extrinsic nervous control. The reticulo-ruminal contractions are not intrinsic and are responses to rhythmic discharges from the brain stem caudal to the intercollicular plane (Clark, 1950; Iggo, 1951). Clark (1953) also showed that the cortex was unnecessary for rumination or movements of the reticulum and rumen; a subcortical area in the brain anterior to the pituitary infundibulum is concerned with these movements.

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## Digestion in Domestic Herbivora

### Part 11

#### The Rate of Passage of Food Residues

This important aspect of the digestive mechanism has recently been re-investigated by Balch (1951) whose paper includes a comprehensive review of earlier work. Balch elaborated the technique described by Lenkeit and Halbeck (1930) who measured the rate of excretion of stained food residues. Balch found that 80% of the food residues were excreted by cows between 70-90 hours after administration and that ground plus unground hay was excreted faster than unground alone. A diet composed solely of ground hay was excreted over a longer period than one of unground hay. These differences were due to changes in the movement of food in the reticulo-rumen. He suggested that a decreased rate of passage through the reticulo-rumen was associated with an increased digestion of crude fibre. Food residues traverse the digestive tract of the horse at almost twice the speed taken in the cow. Using carbon granules as a marker Alexander (1946) found that granules first appeared 22 hours after feeding and were completely excreted in 48 hours. This rate of passage of food in the horse has been confirmed by radiological methods (Alexander and Benzie 1951). Since horses are only about one

third less efficient than ruminants in digesting fibre (Morrison 1950, Armsby 1917), the rate of digestion must be much faster. It seems reasonable, therefore, to suggest that the horse would be a useful experimental animal for the study of cellulose digestion.

Watson and Jarratt (1944) have studied the course of liquids through the stomach of sheep at various ages from birth to maturity and the effect of copper salts on this course. They stated that the oesophageal groove reflex resulted from the mechanical stimulation of the mouth, pharynx or both.

### Products of Digestion

Tappeiner (1884) identified the products of cellulose digestion by rumen bacteria as acetic, butyric and other higher acids. However, the importance of these substances to the ruminant was not fully realised until the last decade. This omission was largely due to the efforts to implicate glucose as the carbohydrate absorbed and metabolised by the ruminant. However, by 1938 Woodman (Woodman and Evans 1938) had abandoned the hypothesis that glucose was the absorbed product of cellulose digestion (Woodman 1930), and confirmed the earlier worker's finding that the lower fatty acids were the principal fermentation products



formed from cellulose by rumen bacteria.

McAnally and Phillipson (1942) then showed that blood from the ruminal, reticular, omasal and caecal veins contained more volatile fatty acid than the peripheral blood and that starch, sucrose and glucose were fermented with the production of the same acids. With sucrose and glucose there was a transient appearance of lactic acid. It is of interest to note that Johns (1951) recently isolated an organism from the rumen which fermented lactate with the production of propionate. The same organism (*Veillonella gazogenes*) occurs in the horse's colon (Alexander, MacPherson and Oxford, 1952).

The clearance from the blood of injected acetate, propionate and butyrate has been studied by Jarrett, Potter and Filsell (1952) who found that the concentration in the blood of propionate fell much more quickly than that of acetate or butyrate. Of these three fatty acids only acetate failed to increase the concentration in the blood of glucose, lactic or pyruvic acids.

Propionic acid being a 3-carbon compound is probably a precursor of glycogen. This is supported by the work of Jarrett and Potter (1950) who found that, in sheep, only propionate increased the concentration in the blood of sugar, pyruvate and lactate. Reid (1950) found that injected propionate relieved insulin hypoglycaemia in sheep as quickly as the injection of glucose. Further



evidence of the glycogen-forming property of propionate was provided by Hitchcock and Phillipson (unpublished) who found that phlorridzinised sheep excreted equivalent amounts of glucose in the urine. Sijpesteijn and Elsdon (1952) have shown that rumen bacteria can convert succinate into propionate.

The metabolism of the small amount of butyric acid formed in the rumen may be accounted for in part by the formation of ketone bodies by the rumen wall (Pennington 1952); the remainder probably forms carbohydrate (Buchanan, Hastings and Nesbett, 1943). Potter (1952) found that intravenous injections of butyrate relieved hypoglycaemia in ewes, lambs and rabbits.

From the work of Popjak, Folley and French (1949) it appears that the large concentration of low molecular fatty acids in cow's milk fat is synthesised in the mammary gland mainly from the acetic acid in the blood. This has been confirmed by perfusing the isolated bovine mammary gland with heparinised blood containing labelled acetate (Cowie et al., 1951). These workers also found that acetate carbon was incorporated into lactose by some mechanism other than  $\text{CO}_2$  fixation, and that cholesterol was synthesised in the udder. Reid (1950) has shown that 90% of the volatile acid in sheep's blood is acetic. The role of the lower fatty acids in milk secretion has been reviewed by

Folley (1949). Folley and French (1949,1950) compared the utilisation of acetic acid in the presence of glucose by mammary tissue from the cow, goat, sheep, rabbit and rat. The ruminant tissues metabolised acetate much better than those of non-ruminants. The arterio-venous difference in acetic acid concentration observed by Reid (1950) between the carotid and jugular blood showed that acetate was used by the tissues of the head. Villee and Hastings (1949) found that the rat diaphragm metabolised acetate and this metabolism was unaffected by insulin. Further, Lipson, Omach's and Carest (1951) using labelled acetate, demonstrated the utilisation of acetate by the dog's gastrocnemius muscle. The acetate metabolism was increased by stimulating the muscle, and acetate did not enter the muscle glycogen. Acetate metabolism has been fully reviewed by Bloch (1947).

It appears from the work of Greeley (1947) that in the goat insulin mediates the storage of glycogen. He found also that after removal of the pancreas, fasting did not cause ketosis.

The heat increment produced by cows after feeding exceeded the basic heat production by 50-100% (Ritzman and Benedict, 1938). These authors considered it unlikely to be the result of fermentation since it also occurred in the horse and elephant. They supposed that considerable time had to elapse in the horse before food

reached the large intestine. However, an increase in fatty acids in the horse's caecum has been found within 3 hours of feeding, (Alexander, 1952). Further, Alexander and Benzie (1951) demonstrated radiologically the rapid propulsion of barium through the stomach and small intestines of the weaned foal, reaching the caecum within 2 hours of feeding. The objections raised by Ritzman and Benedict (1938) to attributing the heat increment after feeding in the horse to fermentation are, therefore, no longer valid. The heat increment of feeding in ruminants is due to the quantitative importance of acetic and butyric acids as products of digestion (McClymont, 1952). McClymont considers the high specific dynamic action of acetic acid due to its being non-glycogenic, not used for protein synthesis and being oxidised by most tissues; it is not stored. Nitrogen metabolism in the ruminant has been reviewed by Krebs (1937) and McNaught and Smith (1947). The evidence for the conversion of ammonia nitrogen to protein has increased. Mills, Lardinois, Rupel and Hart (1944) confirmed the necessity of ample starch for this protein synthesis and found it superior to simple sugars. This may explain the significance of the holotrich ciliates, among the most obvious of the micro-organisms in the sheep's rumen, which Oxford (1951) has shown to be capable of making a

glucosan from glucose, fructose and sucrose. This glucosan has been isolated from protozoa withdrawn from the rumen. It has also been found that if ammonia is to be converted into protein, the protein content of the ration must be low (Wegner, Booth, Bohstedt and Hart, 1941a, 1941b).

Additional evidence of the synthesis from urea and subsequent utilization of amino-acids has been provided by Loosli, Williams, Thomas, Ferris and Maynard (1949a). These authors also found that sheep and goats produced similar amino-acids from three different diets (1949b). In connection with the feeding of urea, it is of interest to note that Clark, Oyaert and Quin (1951) found that the introduction of urea into the rumen inhibited motility. An inhibition lasting 3-48 hours was produced by 10-20 gm., the smaller dose being effective on a poor diet and sometimes spasms and collapse were produced. Comparing the substitution of urea for blood-meal in lactating cows Owen, Smith and Wright (1943) calculated that the efficiency of utilization of urea nitrogen was 75% that of blood-meal nitrogen. Thomson, Graf, Eheart and Holdaway (1952) found that urea and cottonseed meal were of comparable value for milk production and the addition of 22 g. methionine per cow to the diet had no effect.

Shazly (1952a) determined the concentration of

the various volatile fatty acids and of ammonia in the rumen at various times after feeding. An increased concentration of ammonia was correlated with increased concentrations of isobutyric and  $C_5$  acids which he considered to arise from microbial attack on protein in the rumen. In a further paper (Shazly, 1952b) it was shown that the main products of the action of rumen micro-organisms on casein were ammonia,  $CO_2$  and volatile fatty acids. The power of the rumen micro-organisms to deaminate amino acids depended on the animal's diet. McDonald (1952) found that the solubility of the protein determined the rate at which it was attacked by the rumen organism, zein, for example, being relatively insoluble and only slowly digested. He found when casein was fed the main non protein nitrogen in the rumen fluid was ammonia. The ammonia produced by the determination of protein is utilized by rumen organisms to build microbial protein. However, this may be a wasteful process for Chalmers and Cuthbertson (1950) showed that casein was utilized better when introduced directly into the duodenum than when fed into the rumen. Further, Bonnazzi (1951) found that the horse, a colon fermenting herbivore, digests more protein than the cow. However, the importance of the nature of the protein fed to ruminants is shown by Chalmers and Synge (1950) who found a greater retention of nitrogen



when herring meal was fed than when casein formed the protein supplement. Lewis (1951) found that nitrate was reduced in the rumen to ammonia and that sodium nitrate or nitrite in the rumen gave rise to methaemoglobinaemia.

Since methionine is essential to man and the common laboratory animals and as wool contains a large proportion of cystine, the sulphur requirements of ruminants are of great interest. Ferrando (quoted by Owen 1951) maintained sheep in health on diets containing thioglycollic acid. This substance cannot be substituted for cystine in the rats' diet. Further, Reid, Moir and Underwood (1949) have provided some evidence that the sheep can obtain methionine microbiologically. Recently Thomas, Loosli, Williams and Maynard (1951) showed that sulphur deficiency could be produced in sheep and when this occurred urea nitrogen was not utilized and wool growth decreased. It is now clear that ruminants can utilise inorganic sulphur to synthesise cystine and methionine. Block and Stekol (1950) fed sodium sulphate containing radioactive sulphur to cows, collected the milk for several days and isolated the proteins. Cystine and methionine containing radio-active sulphur were isolated in appreciable amounts. Further, Hale and Garrigus (1953) showed the synthesis by sheep of cystine from elemental sulphur and



sulphate sulphur.

Digestion in the caecum and colon has been studied by Trautmann and his collaborators using a histological technique. Trautmann and Ascher (1941a) examined microscopically strips of onion epidermis after suspension for different periods in the goat's caecum. They were only able to detect digestion after the onion epidermis had been in the caecum for a period of 8 hours, and, since they regarded 3 hours as the normal time for digesta to stay in the caecum, the caecal digestion of cellulose was considered to be of little importance. In another paper the same authors (Trautmann and Ascher, 1941b) showed that the treatment of the onion epidermis with dilute acid or carbonate before introducing it into the caecum, improved caecal digestion, although they still regarded it as negligible. However, more recent work (Gray 1947) showed that 30% of the digestible cellulose in the diet was digested in the caecum and colon. The importance of the large intestine as a site of fermentation is well demonstrated by Elsdon et al. (1946); these authors found large amounts of volatile fatty acid in the caecum and colon of many species including the horse and ruminant. Relative to the size of animal they found greater amounts in the horse than in the ox. Alexander (1952) determined the total fatty acid concentration in the

horse caecum and colon under different conditions. He found the concentration of fatty acid decreased on withholding food and increased with feeding. The separation of the component acids showed an interesting difference between the mixture in the ventral colon and that in the dorsal colon. More propionic acid appeared to be produced in the ventral than in the dorsal colon, and more butyric and higher acids in the dorsal than in the ventral colon. The reason for this difference may be that the substrate differs in the two sites of fermentation, the more soluble material being fermented in the ventral colon and in this connection it is interesting to note that the dorsal and ventral colons of the horse support entirely different populations of ciliate protozoa (Adam, 1951). The microbiological activities of the large intestine are clearly worth further study.

The digestion of fat by ruminants has not been studied to any great extent. Aylward, Blackwood and Smith (1937) fed iodised fat to cows and found its absorption slow and continuous, the blood concentration reaching a maximum 36-48 hours after feeding, and falling gradually for five days. In a study of the blood constituents after feeding fat, only lipin and acid - soluble P showed a change whilst lipin P was decreased by fasting (Aylward and Blackwood, 1936). Huff, Waugh and Wise (1951) have

shown that the absorption of lipids by calves is assisted by the presence of glycerol-monostearate.

There can be little doubt that important diseases in ruminants and horses are caused by abnormal fermentations, tympanitis in cattle and some colics in the horse, are obvious examples. These provide additional reasons for the study of fermentative digestion. Some studies of pathological fermentations have already been made. Hungate et al. (1952) found that feeding excess grain or introducing quantities of glucose into the rumen changed the microbial population. This treatment decreased the numbers of cellulytic organisms, increased the Gram + organisms and killed the protozoa. The total volatile fatty acid concentration decreased, motility was inhibited and death occurred in a high proportion of animals. Clark and Weiss (1952) consider bloat in cattle due to the operation of several factors together such as frothing combined with over-filling and impairment of the ruminal reflexes. No experimental study of colic in the horse has yet been made.

### Absorption

It has been known for many years that certain drugs such as pilocarpine and atropine are absorbed through the rumen epithelium (Trautmann, 1933). However, it was not until 1944 that Barcroft,

McAnally and Phillipson found that in sheep at least 1-5 gm. of volatile fatty acid were absorbed every hour by this route, clearly showing that these important products of carbohydrate fermentation entered the body through the rumen. It has since been shown that sodium (Danielli et al. 1945) ammonia (McDonald, 1948), sodium orthiodohippurate (Barcroft et al., 1944), hydrocyanic acid (Coop and Blackley 1949) and inorganic phosphate (Scarisbrick and Ewer, 1951) are absorbed from the rumen. The permeability of the rumen epithelium has been studied in some detail by Parthasaraphy and Phillipson (1953) who found that sodium and potassium were absorbed when the concentration in the rumen liquor exceeded that in the blood. Mercuric chloride did not affect the absorption of these two ions although it inhibited the absorption of chloride and acetate. In a further study of rumen absorption Masson and Phillipson (1951) found that an appreciable quantity of the butyrate leaving the rumen did not enter the blood draining the organ. This has been explained in part by some experiments of Pennington (1952) who found that rumen epithelium in vitro could metabolise butyrate, about 50% of which could be accounted for by the ketone bodies formed. Masson and Phillipson (1951) also found that the rate of absorption of acetate was related to the concentration in the arterial blood. These

authors noted the appearance of chloride and carbon dioxide in the rumen when aqueous solutions of these lower fatty acids were in that organ.

Support for the idea of the omasum being entirely concerned with the absorption of water has been provided by Garton (1951). He found evidence for the concentration of phosphate, magnesium and calcium in this organ. It is also of interest to note that the amount of volatile fatty acid leaving the abomasum is much less than is absorbed from the rumen (Phillipson et al., 1949), and that less than 6 gm./day of reducing substances enter the ileum (Heald, 1951).

The sole experiment so far discovered on absorption from the equine stomach is that of Colina (1886) who demonstrated that strychnine was not absorbed from this organ. There is evidence for the absorption of glucose from the horse ileum (Alexander 1954) and for the absorption of fatty acids from the large intestine (Barcroft et al, 1944).

The absorption of volatile fatty acids from the ruminant caecum has been demonstrated (McAnally and Phillipson, 1942) and absorption of water from the caecum and colon studied (Trautmann and Ascher 1939). These latter authors found that the quantity absorbed depended on the total amount of sodium chloride in solution and not on the concentration. The quantities of sodium sulphate and magnesium



sulphate required to cause diarrhoea were much greater than the amount of sodium chloride which produced the same effect. Sporri and Ascher(1940), however, considered the spiral colon to be the main site of water absorption in ruminants although all parts of the large bowel carry out this function.

### Secretions

The most studied secretion of the digestive tract and associated glands of the ruminant has been the saliva. Trautmann and Albrecht (1931) measured the secretion of parotid saliva in goats; the adult goat secreted 0.4-18.5 ml. in 5 min., kids secreted only one-tenth of this. Sharma (1936) found the composition of bovine saliva as follows: S.G. 1,008, pH 8.8, no mucin or ptyalin, water 99.15%, organic matter 0.17%, inorganic 0.68% Na 0.2768, Mg. 0.006%, chlorides 0.0154%, sulphates 0.0145% and phosphates 0.359%. A more comprehensive study of the composition of sheep mixed saliva has been made by McDougall (1948) (dry matter 1.0 - 1.4 gm./100 ml., ash 0.7 - 0.9 g., Na. 370 - 462mg., K 16.46 mg., Ca 1.6 - 3.0 mg., Mg. 0.16 - 1.0mg., P 37 - 72mg., chloride 23 - 43 mg., CO<sub>2</sub> 117 - 283 ml. pH 8.23). He found the rate of flow varied between 930 - 1840 ml./day from one gland and was increased by eating and rumination. Reid and Huffman (1949) found bovine saliva had a pH of 8.53 and contained 0.15 mg. ascorbic acid forming about



80% of the rumen ascorbic acid. It had a low surface tension. This latter fact probably accounts for its efficiency as a "wetting" agent.

The rumen, reticulum and omasum are devoid of glandular epithelium, the only secretory part of the ruminant stomach is the abomasum. Sheep (Popov, 1932), goats (Bickel, 1905) and calves (Espe and Cannon 1937) secrete continuously an acid juice from the abomasal epithelium. This continuous secretion was maintained in the absence of vagal fibres (Popov, 1932). A further difference between ruminants and animals such as dog and man has been provided by Hill (1952). This worker found that insulin decreased the volume and acidity of the abomasal secretion in goats with innervated abomasal pouches. This decrease was unrelated to the degree of hypoglycaemia. In a study of abomasal secretion Kuimov (1952) found a depression after giving copper or sodium sulphate and a stimulation from feeding green oats or fresh hay. Florey et. al. (1941) found the juice from duodenal fistulae of sheep and goats thicker than that from the pig, cat or dog and contained much mucin. The sheep juice had a pH of 8.3 - 8.4; in goats, feeding caused a flow of juice and this species secreted as much as 150 ml. in 7 hours. So far as can be ascertained the secretions of the horse's small intestine have not been investigated. However, an interesting observation has been made on the large intestine, Alexander

et al. (1952) noticed a muco-protein like substance in the liquor of the dorsal colon, which, since it was absent from the ventral colon liquor, must have been secreted within the dorsal colon. Histological confirmation of this deduction has been provided by Morris (1954) who found goblet cells in much greater numbers in the dorsal than in the ventral colon. Wright et al. (1940) studied the enzymes in duodenal juice from the goat and found no protease, no peptidase, phosphoesterase which hydrolysed ribonucleic acid and adenosine deaminase in the ileal mucosa of the calf.

The larger part of the food of horses and ruminants is reduced to molecules capable of absorption and metabolisation through the agency of micro-organisms. So far the enzymes associated with this microbial digestion have attracted little attention. However, Hungate (1943) claims to have shown the presence of a cellulose in *Diplodinium*, a ciliate inhabiting the rumen of cattle, and enzymes capable of decomposing pheno-phthalein glucuronide have been found in the rumen micro-organisms (Karunairatnam and Levvy, 1951) and in the micro-organisms of the horse colon (Alexander and Marsh, unpublished). It is possible that such enzymes are concerned in the digestion of polysaccharides containing B - linked glucuronic acid residues such as hemicellulose, (Marsh, Alexander and Levvy, 1952).

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SOME OBSERVATIONS ON GENERAL ANAESTHESIA  
IN PONIES

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## SOME OBSERVATIONS ON GENERAL ANAESTHESIA IN PONIES

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### INTRODUCTION

In recent years a variety of new ways of anaesthetising horses have been described; for example Marnac and Lemetayer (1930) reviewed earlier work and described a technique for the use of chloral hydrate, Henkels (1938) devised an apparatus for the administration of ether by inhalation, Francis and Parry (1949) introduced  $\beta$  naphthoxy-ethanol, a new anaesthetic drug, and in 1952 Dyce, Jones and Wadsworth described a method employing cyclopropane. This search for new techniques and new drugs illustrates the difficulties associated with general anaesthesia in the horse.

During the development of techniques for the fistulation of the horse's intestine (Alexander and Donald, 1949; Alexander, 1951, 1952) experience was obtained in anaesthetising ponies for periods of up to three hours. Since opportunities of observing the effects of anaesthesia of this duration are unlikely to be of frequent occurrence in general practice, it was felt that the description of a method of anaesthetisation with pentobarbitone which had proved satisfactory, together with some observations on the use of chloral hydrate and ether for long anaesthesias, would be of interest.

### METHODS

*Pentobarbitone.* A solution of sodium pentobarbitone containing 60 mg/ml (Abbotts Veterinary Nembutal) was given by injection into the jugular vein. Anaesthesia was induced with the animal in a standing position without restraint other than an assistant holding the head. The induction dose was computed on the basis of 20 ml solution per 50 kg and an appropriate number of 20 ml syringes were prepared. The first 20 ml was injected rapidly and the syringe changed; the anaesthetic solution was then injected slowly until the animal subsided to the ground. In animals of 100 to 250 kg. recumbency was produced by between 25 to 40 ml of solution. With the animal lying quietly on the ground the injection of anaesthetic solution was continued until full anaesthesia had been induced.

As the operation progressed, additional doses of pentobarbitone were given. This was facilitated by using an indwelling cannula. The indication for the administration of these additional doses was determined by the animal's response to the operation, the anaes-

TABLE I  
PENTOBARBITONE SODIUM ANAESTHESIA IN PONIES  
DOSE IN MG/KG

Pony	Weight kgs	Induction	Maintenance Time in Minutes after Induction													
			10	20	30	40	50	60	80	100	120	140	160	180	200	210
Ginger Op 1	222	14.4														
Ginger Op 2	222	15.0		3.0		3.0		2.4			1.8					
Ginger Foal Op 1	138	14.5	4.8		4.8											
Ginger Foal Op 2	138	14.5	4.8		4.8			3.4			4.8					
Black Foal	129	20.7														
Judy Op 1	102	19.6	3.3				6.5									
Judy Op 2	154.5	9.5	2.0	2.0	6.0			2.0		4.0				6.5		
Rufus	204	13.1														
Tilly Op 1	167	11.9														
Tilly Op 2	167	17.9	2.0	1.0		1.0					1.0					
Tilly Op 3	198	10.1	3.4	3.4				2.5								
Blackie Op 1	191	24.4	3.5	3.5		3.5										
Blackie Op 2	191	13.9														
Blackie Op 3	176	10.5		3.0				3.0								
Punch Op 1	131	20.4	5.1	5.1		3.7		2.5					1.5	1.5	1.5	1.5
Punch Op 2	131	13.2	2.0	5.0	4.0	3.5		3.5		3.5				3.5		

thetic solution being injected slowly until the required depth of anaesthesia was produced.

The respiratory depression produced by anaesthetic doses of pentobarbitone produced cyanosis of the tissues which it was found advantageous to treat by the continuous administration of oxygen with 5 per cent. carbon-dioxide. This was done by passing a catheter into the nasal cavity, connecting it to a cylinder containing the mixture of gases and allowing a gentle flow of gases to pass into the nostrils. Table I shows the quantities of pentobarbitone required to induce and maintain anaesthesia in sixteen cases.

*Chloral hydrate.* The drug was dissolved in water to make a 10 per cent solution. The initial dose was calculated on the basis of 7 g. chloral hydrate per cwt. body weight and additional injections of 100 ml of the solution were given at intervals to maintain the required depth of anaesthesia.

*Thiopentone/Ether Anaesthesia.* A 10 per cent solution of thiopentone was injected intravenously until recumbency was produced. This required a dose of 20 to 25 mg/kg. With the animal recumbent the anaesthetic apparatus of Henkels (1938) was fitted and the administration of ether started. The rate of administration of ether was adjusted so as to maintain the required depth of anaesthesia.

TABLE II  
DEATHS PROBABLY DUE TO ANAESTHETIC POISONING

	<i>Chloralhydrate</i>	<i>Thiopentone/Ether</i>	<i>Pentobarbitone</i>
Number of Anaesthesias ..	4	4	20
Number of Deaths ..	2	2	0

#### RESULTS

Table II shows the deaths which occurred during or following 28 consecutive anaesthesias. Extracts from the post-mortem reports by Mr. I. S. Beattie of the four anaesthetic deaths are detailed below. The deaths appeared to be attributable to the anaesthetic drug.

##### *Deaths After Chloral Hydrate*

*No. 146/149.* A 5 year old Dartmoor Pony Mare (250 kg) which received a total of 60g chloral hydrate and was under the anaesthetic for three hours died 4 days after the operation. Petechiae were present on the pleura, and the lungs were congested and oedematous. The myocardium showed fatty change. The liver was deep yellow with dark brown mottling, soft and friable. The kidneys were pale, soft and the cortical tubules showed diffuse fatty change. The operation wound was healing normally. Death was probably due to chloral hydrate poisoning.

No. 358/48. An aged Pit Pony Mare which was operated on the 11th November, 1948 and appeared to be progressing well, died suddenly 7 days after the operation. At post-mortem the carcass was in good condition. On opening the abdominal cavity a small quantity of yellowish, turbid, serous fluid escaped and multiple congested tags of an organising peritonitis were observed scattered over the visceral and parietal peritoneum, these lesions becoming confluent and more prominent in the region of the operation wound in the left flank. A large verminous aneurysm with extensive thrombosis involved the cranial mesenteric artery; both kidneys showed infarcts of various sizes probably arising from this thrombosis. The lungs were slightly congested and also presented a number of small infarcts. The myocardium was pale and fatty with a single infarct in the wall of the left ventricle. The liver was pale and mottled.

*Deaths after Thiopentone/Ether:*

No. 63/50. A 7 year old pony mare which was free from clinical signs of disease. A fistula of the colon was prepared which required 6g. Thiopentone to induce, and 1000 ml. ether to maintain, anaesthesia. The operation lasted approximately 2 hours. For three days following the operation the animal was dull and not eating and jaundice appeared on the fourth day. Eight days after the operation the animal became prostrate and was shot. Post-mortem examination showed that the operation wound was healing normally. The lungs were congested and oedematous. The liver, kidneys and heart showed widespread and advanced fatty change probably associated with the toxic action of the anaesthetics.

The second of these deaths was that of a 4 year old Dartmoor pony in good condition with no clinical evidence of disease. Anaesthesia was induced with Thiopentone and continued with ether. There was no undue struggling or excitement. The projected laparotomy was through an incision along the linea alba; however, as soon as the peritoneum was incised the animal collapsed, heart and respirations failing together. No post-mortem examination was made.

DISCUSSION

Marcenac, Carnus and Comby (1935) claimed to have anaesthetised about five-hundred horses with intravenous injections of chloral hydrate without observing any ill-effects. Wright (1952) recorded a single fatality in 300 cases in which chloral hydrate was used as narcotic or anaesthetic. A similar claim has been made for inhalation anaesthesia with ether, Henkels (1938) having anaesthetised five hundred horses in this manner with a complete absence of complications. In view of these claims it was surprising to find these drugs causing deaths in the small number of cases recorded here. The

explanation of this difference was not obvious; it might be a question of total amount of drug used, since most veterinary operations are of short duration whereas the anaesthetics described here lasted up to 3 hours; however, Marcenac and Lemetayer (1930) mentioned operations of 3 to 4 hours duration being conducted under chloral hydrate anaesthesia. The deaths recorded here from chloral hydrate and ether anaesthesia were not due to these drugs being used for the earliest operations in the series and, therefore, due to inexperience, as all three drugs were used with equal frequency in the first twelve operations. It then seemed likely that pentobarbitone had advantages not possessed by the other drugs both of which by this time had been responsible for fatalities.

The toxic effect of chloral hydrate on the myocardium and liver is mentioned by Wright (1952); therefore, the lesions found after anaesthesia with this drug were not unexpected. Although the death of the second pony (146/49) was probably due, in part, to the numerous infarcts, the extensive damage to the liver and heart was probably caused by chloral hydrate and must have been a contributory factor.

Henkels (1938) in 500 horses given ether by inhalation and Longley (1950) in six given ether intravenously encountered no signs of toxicity; it was, therefore, surprising to find a death after ether anaesthesia in which there were extensive pathological changes in the heart and liver. The death which occurred in the early stages of an operation under ether anaesthesia was difficult to explain. In addition to the toxic properties of ether, a further disadvantage of the Henkels apparatus was the unpleasant atmosphere produced by large volumes of ether vapour being blown through the expiratory valves into the theatre. This objection would be reduced when operating in the open.

Although pentobarbitone seemed a safer anaesthetic in ponies than either chloral hydrate or thiopentone/ether, it was not free from objection. The main disadvantage was the length of time taken by the animal to regain equilibrium. After an operation of two hours duration, the ponies were not usually capable of standing until about three hours after the last dose of anaesthetic. The animals appeared conscious before this time but did not regain control of their hind limbs. This objection was equally applicable to chloral hydrate. Excitement during the induction of anaesthesia with pentobarbitone was not observed; however, during the period of recovery there was some struggling especially when the animals attempted to rise. These movements were not difficult to control in ponies but might be a serious objection to the use of this anaesthetic in larger animals.

The respiratory depression and consequent anoxia produced by pentobarbitone was alleviated by the intra-nasal administration of oxygen with 5 per cent carbon-dioxide. However, a further disadvantage common to both chloral hydrate and pentobarbitone when used as general anaesthetics was the lack of muscular relaxation. In laparotomies through the flank this disadvantage was overcome



by blocking the last six intercostal nerves with a 1/1000 solution of cinchocaine.

It was interesting to note that the dose of pentobarbitone required to induce anaesthesia in ponies was about half that used in dogs, (Wright 1952).

#### SUMMARY

Twenty-eight laparotomies on ponies have been carried out under general anaesthesia. Sodium pentobarbitone appeared to be a safer anaesthetic for major surgical procedures than either intravenous chloral hydrate or the combination of sodium thiopentone intravenously followed by the inhalation of either.

The technique of anaesthetisation with sodium pentobarbitone has been described.

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## CAECOSTOMY IN THE HORSE

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### INTRODUCTION

ALTHOUGH the digestive system is frequently involved in disorders of the horse, there is little information available about the mechanics of the gastro-intestinal tract in this species. Indeed, other than the early observations of Colin (1886) and the studies on stomach movement of Schalk and Amadon (1921), the problem seems to have attracted few students. The size and inaccessibility of the abdominal organs and the difficulties attendant upon surgical intervention in this region have undoubtedly deterred such investigations. With a view to overcoming some of these difficulties, the possibility of preparing permanent fistulae into the large intestine was explored, and a satisfactory technique evolved.

### TECHNIQUE

The operation was performed in two stages. The first involved the formation of a peritoneal ring and the exteriorisation of a portion of the caecum; the second, performed at a later date, entailed the opening into the caecum and the completion of the fistula.

*Pre-operative Treatment.*—Food was withheld for the 36 hours prior to operating and no water given in the last twelve hours of this period. An extensive area on the right flank was shaved, thoroughly washed and cleansed with spirit 24 hours before the operation.

*Restraint.*—The animal was cast on its near side with side-lines and the anaesthetic solution injected into the right jugular vein by gravity. When anaesthesia was complete, both fore and the near hind legs were secured together, and the off hind drawn backwards with a single rope. This position was maintained until the final stage of the operation that of suturing bowel to skin, when the off hind leg was drawn forwards and secured to the others.

*Anaesthesia.*—Chloral hydrate, made up in 10 per cent. solution with 1 per cent. sodium citrate, was used to induce and maintain anaesthesia, the initial dosage being calculated on the basis of 7 g. chloral per cwt. body weight. Further injections of 100 c.c. were given when deemed necessary to maintain the required depth of anaesthesia.

### *Operation*

The site was swabbed with spirit, then with iodine solution, and towels applied. An incision was made through skin and subcutaneous tissue, commencing 2 in. below the mid-point of the line joining the coxal tuberosity to the head of the last rib and extending downwards and slightly forward for a distance of 6 in. Towels

were then applied to the lips of the wound and the incision deepened through the aponeurosis of the external oblique abdominal muscle, exposing the thick internal oblique abdominal muscle. This muscle was incised across its fibres in line with the primary incision. A considerable oozing of blood resulted, but only one major vessel, a branch of the circumflex iliac artery, was encountered. The lips of the wound were then strongly retracted to expose the fascia of the transverse abdominal muscle. The fibres composing this fascial sheet were split with scissors to within  $\frac{1}{2}$  in. of the commissures and the exposed peritoneum secured by Allis forceps at the top and bottom of the incision and two points midway; following traction on the central pair of forceps, the peritoneum was opened along the line of the wound.

The next step was to form a peritoneal ring by suturing the peritoneum to the outer edge of the internal oblique muscle. Interrupted sutures of 00 silk on straight needles were placed  $\frac{1}{4}$  in. apart. A good "bite" of peritoneum and muscle was taken and special attention was paid to the upper and lower commissures to ensure their being fully covered.

The caecum was then located and completely withdrawn from the abdomen to ensure identification. An area on the lateral surface at the junction of the base and the body, was selected for exteriorisation, the remainder of the organ being returned to the abdomen. The retained portion was firmly secured to the peritoneal ring by interrupted sutures of 00 silk placed  $\frac{1}{2}$  in. apart, care being taken to obtain good apposition of peritoneal and serosal surfaces. The sutures included the caecal mucosa within their grasp and were so placed that a considerable portion of the caecal wall protruded from the lips of the wound. As before, the commissures received special attention. Finally the caecum was sutured to the lips of the skin wound by through and through mattress sutures of heavy linen. The serosal surface of the caecum was thus brought into contact with edges of the skin wound.

The area was thoroughly smeared with sterile vaseline and a similarly treated Gamgee pad stitched over the exposed bowels. This dressing was changed daily until the second stage of the operation.

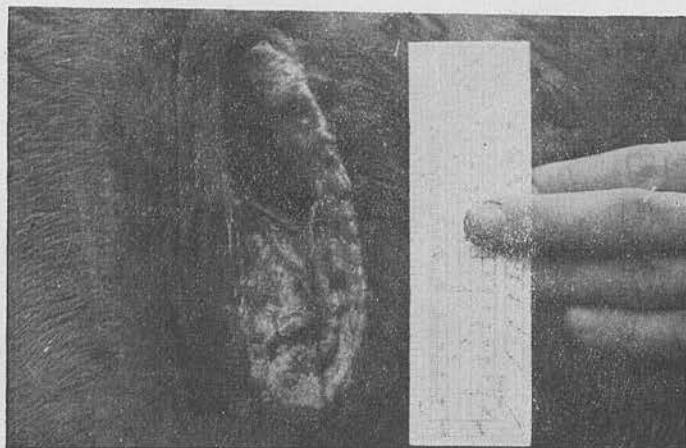
*Second Stage.*—A considerable reaction took place on the exposed serous surface, with marked exudation and great thickening of the bowel wall. Granulation tissue soon appeared, and after five and a half days the serosal surface was firmly adherent at most points to the edges of the skin wound. The second stage was then undertaken.

General anaesthesia was again induced with intravenous chloral hydrate and the animal secured in a recumbent position. The whole area was then thoroughly washed with soap and water, swabbed with normal saline solution and cloths applied. After the removal of excessive granulations, the caecal wall was picked up with forceps and slit

(a)

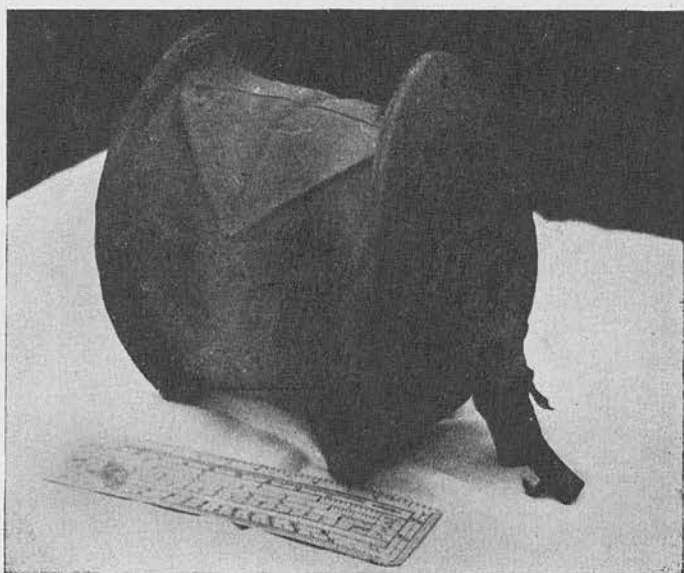


(b)

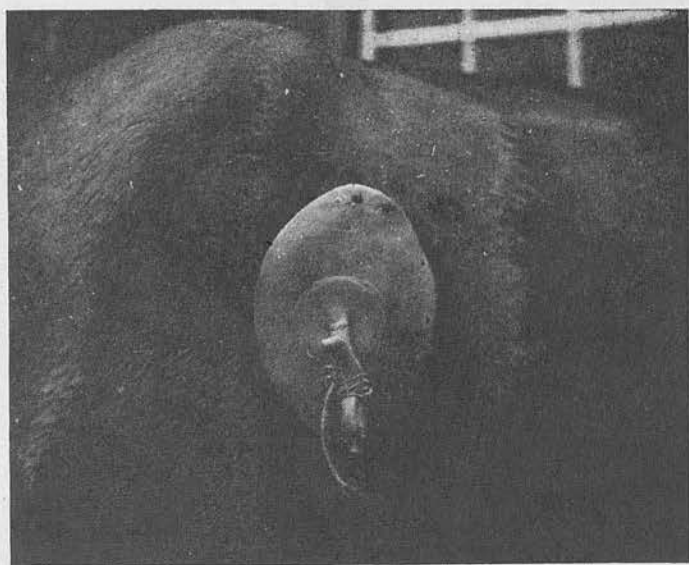


(c)

FIG. 1.—(a) Caecum exposed at operation. (b) Caecum exteriorised. (c) Fistulae three months after completion.



(a)



(b)

FIG. 2.—(a) Inflatable rubber stopper. (b) Stopper *in situ*

with scissors along its midline to within  $\frac{1}{4}$  in. of upper and lower commissures. A moistened towel was then quickly inserted to pack off the caecal contents. Close apposition of the lips of the caecal wound and the skin edge was then obtained, using interrupted sutures of No. 7 nylon. A firm hold of skin and caecal wall was taken, the sutures being placed  $\frac{1}{2}$  in. apart. Hot packs wrung out with saline were applied constantly throughout. The special "stopper" was next inserted and securely seated. It was inflated to moderate tension and the edges of the outer plate attached to a surcingle passing round the back and loins.

At first there was a good deal of inflammatory reaction around the fistula with considerable serous exudation and formation of granulation tissue. Owing to leakage of caecal contents it was impossible to avoid contamination of the wound and some surface infection resulted. By the seventh day from completion of the second stage, the mucosal surface had firmly united with the skin edge at all points. From then on the reaction quickly subsided, the mucous membrane showing a moist healthy surface. Three weeks from completion of the second stage, the epithelium at the fistula edges was commencing to show a squamous character.

A slight post operative rise in temperature and pulse rate was noted 48 hours after the first stage, but this had subsided by the 72nd hour. At no other time was the temperature or pulse abnormal, nor did the animal refuse food. Various steps of the operation are illustrated in Figs. 1 and 2.

#### THE INFLATABLE STOPPER

The form of apparatus finally employed for closure of the fistula consisted essentially of two lozenge shaped soft rubber plates joined by an oval inflatable tube. A photograph of the apparatus is shown in Fig. 2. The outer plate measures 8 in. in its vertical diameter and 6 in. in its greatest horizontal diameter and is moulded in  $\frac{1}{4}$  in. rubber. The inner plate is 1 in. less in overall measurement and of the same material. The distance between the two plates is 3 in. The inflatable tube is attached to the plates  $1\frac{1}{2}$  in. from their outer margins. For experiments which entail frequent manipulations of the fistula as, for example, the hourly sampling of caecal contents, it is convenient to have the stopper fitted with a central tube connecting the rubber plates and closed with a rubber stopper.

#### DISCUSSION

The problem presented two main aspects, the exteriorisation and opening of the large bowel without peritoneal soilage and, having made a fistula, the designing of some means of preventing leakage of ingesta. The surgical technique entailed fewer difficulties than was at first expected in view of the current teaching regarding abdominal surgery in the horse. Chloroform anaesthesia was used



for the first experiments, but prolonged anaesthesia necessary for the operation caused pneumonia; intravenous chloral hydrate anaesthesia avoided this complication. A starvation period of 36 hours was found to be the minimum time necessary to obtain a relatively empty caecum.

It was considered that the main factor in preventing peritoneal contamination was the careful preparation of the peritoneal ring so as to secure adhesion between serosal and peritoneal surfaces. The horses tolerated this interference surprisingly well and, in recent experiments, the provision of a fistula has not caused the animal to miss a single feed.

The opening into the caecum should be delayed until the reaction has subsided and complete union achieved between the opposed surfaces: this was usually found to have occurred by the fifth or sixth day. Much more difficulty was encountered in designing a suitable apparatus for closing the fistula. In the early experiments a large tracheal cannula was used, but this only allowed a relatively small opening and permitted extensive leakage. Further experiments with a variety of metal cannulae of this basic design proved unsatisfactory. The idea of having a solid type cannula was abandoned and an inflatable rubber stopper devised. The design finally selected closed the fistula effectively, prevented leakage of the air and ingesta and did not damage the mucosa. Horses fitted with this type of apparatus showed no loss of condition over several months.

Fistulae of the type described should prove valuable in physiological and pharmacological studies. The rubber stoppers could be modified for various species and should prove more satisfactory than the conventional types.

#### SUMMARY

A method has been described for the preparation of permanent fistulae into the large intestine of the horse.

An inflatable rubber stopper was devised which prevented leakage from the fistula.

#### ACKNOWLEDGMENTS

We wish to take this opportunity of thanking Mr. and Mrs. Allison, of Norton Mains, for the facilities to conduct these experiments, Mr. Robin Allison for the care taken to secure the photographic records and the Agricultural Research Council for a grant to one of us (F.A.) which allowed this work to be performed.

The inflatable stoppers were made by the North British Rubber Company, Edinburgh, and we are grateful for the interest shown by Mr. McGowan of this company.

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A RADIOLOGICAL STUDY OF THE DIGESTIVE TRACT OF  
THE FOAL. By F. ALEXANDER and D. BENZIE. From  
the Rowett Research Institute, Bucksburn, Aberdeenshire.

*(Received for publication 11th January 1951.)*

THE topography of the digestive tract of the horse has been extensively studied by the dissection of preserved specimens [Ellenburger and Baum, 1926; Bradley, 1946], and although this method has provided much valuable information, it was felt that the application of radiological techniques to this species would give information about the disposition of the digestive tract under more normal conditions. So far as could be ascertained, radiological studies on the horse have been confined to the bones of the extremities, and the purpose of the present investigation was to apply radiographic techniques to anatomical and physiological studies of the horse's digestive tract. The opportunity was taken to determine the rate of passage of a barium meal through the digestive tract.

#### METHOD.

The observations described were made on two pony foals during the suckling period and after weaning at the 4th month. At weaning, the foals were given a diet of cut grass and oats.

Preliminary attempts at radiographing the foals showed that the dorso-ventral position gave the best topographical information. In the preliminary attempts there were two principal difficulties in producing a good radiograph of the digestive tract: the respiratory movements of the animal and scattered radiation. The same principles were adopted as those used on the pregnant ewe [Benzie, 1951]. Loss of definition due to respiratory movements was reduced by keeping the exposure time short, and secondary radiation by placing a moving grid in front of the film, and confining the primary X-ray beam to narrow limits by means of a localising cone.

A 4-valve high-voltage generator with a rotating anode X-ray tube capable of carrying the required mA and kV was used in conjunction with Ilford intensifying screens and Philips Potter-Bucky diaphragm. An exposure of 0.8 second at 200 mA and 80 kV with the tube 3' 6" distant from the animal was given for 6-month-old foals weighing about 120 kg. The Potter-Bucky diaphragm was essential to produce reliable radiographs; hence it was necessary to use animals small enough to lift on to a standard pedestal diaphragm. If work on larger animals is contemplated it will be necessary to design a suitable diaphragm.



The barium meal given to the suckling foals consisted of 35 g. barium sulphate suspended in 200 ml. of cow's milk, to which was added 16 g. of sucrose. This contrast meal was given after warming to 37°C., and little difficulty was experienced in persuading the foals to drink this mixture from a bottle. After weaning, the barium meal consisted of 100 g. barium sulphate, 35 ml. milk (cow's), 10 g. sucrose and 30 g. crushed oats, mixed to a thick paste, and was put into the mouth with the aid of a spatula. Two series of radiographs, extended over a 48-hour period, were made of each animal, both before and after weaning.

## RESULTS.

### *Topographical Observations.*

*Stomach.*—This organ lay on the left side of the median plane covered by the 11th–15th ribs. The position varied between ribs 10–17, probably depending on whether the picture was taken at inspiration or expiration, and also on the activity of the organ. The appearance of the stomach was remarkably constant in both the suckling and weaned foal (figs. 1 and 2). In the majority of plates showing the stomach a gas bubble was present along the caudal border. This was identified as the saccus cæcus (fig. 1b).

*Duodenum.*—The position of the beginning of the duodenum was constant and lay in the midline. The plates suggested that the position of the pylorus was to the right of the main mass of the stomach and directly underneath the vertebral column. The pyloric antrum was regarded as the right and inverted part of the U-shaped shadow in fig. 1. The sigmoid portion of the duodenum was clearly seen in both milk-fed and weaned foals (figs. 2 and 2a). In the milk-fed foal the second part of the duodenum was distinguished up to the second flexure.

*Small Intestine.*—The position of this part of the digestive tract was very variable, but seemed in the main to occupy the left side of the median plane (figs. 3 and 3a).

*Cæcum.*—This structure was not seen in radiographs of the milk-fed foal (fig. 4) but was distinct in the weaned animal. The cæcum lay obliquely across the right half of the abdomen, with the widest part mostly caudal and the apex cranial and on the level of the 15th thoracic vertebra. The characteristic conical shape of the organ facilitated its identification (fig. 4a).

*Ventral Colon.*—In the milk-fed foal the ventral colon was not so clearly defined and had few haustra; the right ventral colon was more easily identified than the left. In the weaned foal the right and first part of the left ventral colon was clearly seen and showed the typical haustra. The whole ventral colon appeared larger in the weaned than in the unweaned animal. The pelvic flexure was not shown (figs. 5 and 5a).



*Dorsal Colon.*—The left and right dorsal colons were visible in both weaned and unweaned foals, but in the unweaned animal appeared to occupy much less of the abdominal cavity and, on the right side, did not extend caudally beyond the 17th thoracic vertebra. The dorsal colon of the weaned foals showed fewer haustra than the ventral colon.

*Small Colon.*—The small colon for the most part occupied the left caudal part of the abdomen. However, this organ was sometimes seen on the right side of the median plane. It was easily identified by the characteristic form of the scybala (fig. 6a). The rectal ampulla, when full, was clearly defined (fig. 6).

#### *Rate of Passage of Barium Meal.*

The observed rate of progress of the barium through the digestive tracts of unweaned and weaned foals is shown diagrammatically in fig. 7.

*The Suckling Foal.*—The most outstanding feature noted during the progress of a barium meal through the digestive tract of the milk-fed as compared with the weaned foal was the longer delay in the stomach and ileum. The cæcum was never seen as a definite structure. Since barium was noticed in the right ventral colon little more than an hour after feeding, whilst the stomach and ileum still contained a large part of the meal, the cæcum was probably obscured by the ileum and large colon. The barium did not stay long in the ventral colon, but was stored in the right dorsal colon and small colon until excreted.

*The Weaned Foal.*—In foals on a diet of cut grass and oats the barium meal passed very quickly through the stomach and small intestine to reach the cæcum and right ventral colon; 30 minutes after the barium meal a considerable amount of barium was present in the ileum (fig. 2a). The cæcum was outlined as a distinct entity, in contrast with the suckling foal, and occupied an appreciable part of the abdomen. The ventral colon after weaning showed well-marked and characteristic haustra, and the barium meal was seen slowly advancing from the right to the left ventral colon (fig. 5a). The digesta seemed to be subjected to a thorough mixing in this organ. The haustra were more clearly defined than in the suckling animal.

#### DISCUSSION.

The large size of the intestinal mass in the horse makes it difficult to appreciate its disposition in preserved specimens, and, since the intestine is composed in the main of plain muscle in a state of tone which is lost in the dead animal, it is not unlikely that this may affect the relations of the intestine. Whilst the radiographic method is ideal in giving a picture of the barium-filled intestine in life, it is limited in only

providing a shadow projection in one plane, and sometimes the overlapping of shadows make the interpretation difficult.

The disposition of the intestine, as determined radiographically, agreed closely with the description of the dissected specimen [Bradley, 1946]. The stomach was easily identified, and its relations to the ribs were as described by Bradley [1946]. The duodenum, because of its fixation, was similarly not difficult to identify, whilst the remainder of the small intestine did not present any noteworthy anatomical features. However, the radiographs of the large intestine proved particularly interesting, especially when the radiographs of the same foal were compared before and after weaning. The cæcum in the suckling foal was not seen as a distinct entity, whereas in the weaned foal this organ was clearly defined and, because of its characteristic shape, easily identified. The most probable explanation of this interesting observation seemed to be that, in the suckling foal, the cæcum was obscured by the mass of small intestine and ventral colon. This conjecture was supported by the fact that the barium meal remained longer in the stomach and small intestine of the suckling foal than in the weaned foal. Further, the ventral colon of the suckling foal emptied more quickly than that of the weaned foal, and it seemed reasonable to suppose that the cæcum would behave in a like manner. Hence, by the time the ileum of the suckling foal was clear of barium, insufficient remained in the cæcum to define the organ. It was also interesting to note that the ventral colon of the suckling foal did not show the numerous haustra which distinguished this part of the large colon in the weaned animal. Since the digestion of milk is presumably completed in the stomach and small intestine, the suckling foal has little need of large fermentation chambers in the digestive tract. However, as the weaned foal ingests a considerable amount of food-stuffs which need to be fermented before becoming available to the animal, it must use its capacious cæcum and colon for this purpose, and the numerous haustra seen in the ventral colon of the weaned foal may be evidence of its functional activity. Further experiments are necessary to determine the amount of fermentation proceeding in the various portions of the large intestine. The small colon varied in disposition, as might be expected in view of the loose mesocolon attached to this part. It was disposed for the most part on the left side of the abdomen, but frequently scybala characteristic of this organ were seen on the right.

In addition to the topographical information provided by this investigation, the serial radiographs gave a picture of the progress of the barium along the digestive tract. The time taken for barium to traverse the alimentary tract agreed reasonably well with that determined by markers such as carbon particles [Alexander, 1946], and it was interesting to find that the overall rate of progress did not differ greatly before and after weaning.

SUMMARY.

1. The disposition and surface relations of the alimentary tract of the foal have been studied radiographically.

2. The rate of progress of a barium meal through the digestive tract was determined. The barium traversed the stomach and ileum of the weaned foal faster than that of the suckling foal, but was longer retained in the cæcum and ventral colon of the weaned foal.

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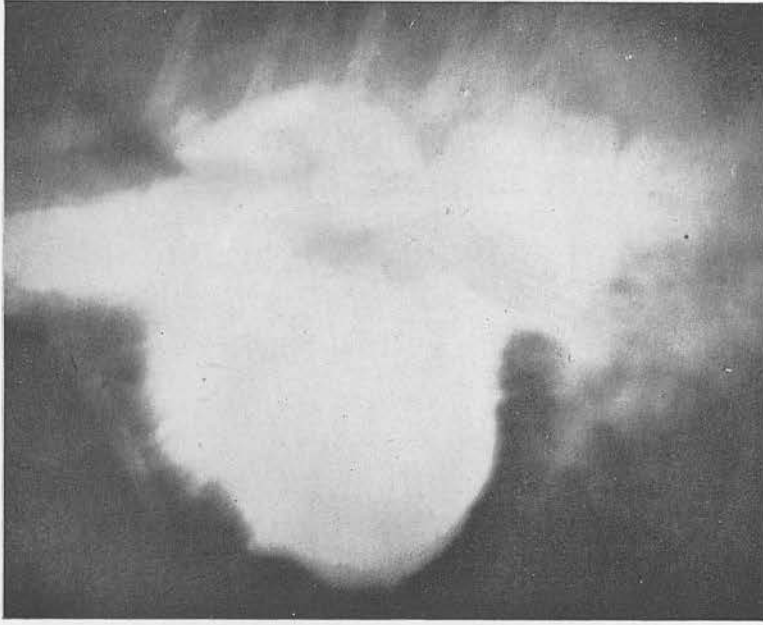


FIG. 1a.—Dorso-ventral view of stomach in weaned foal 5 minutes after barium meal.

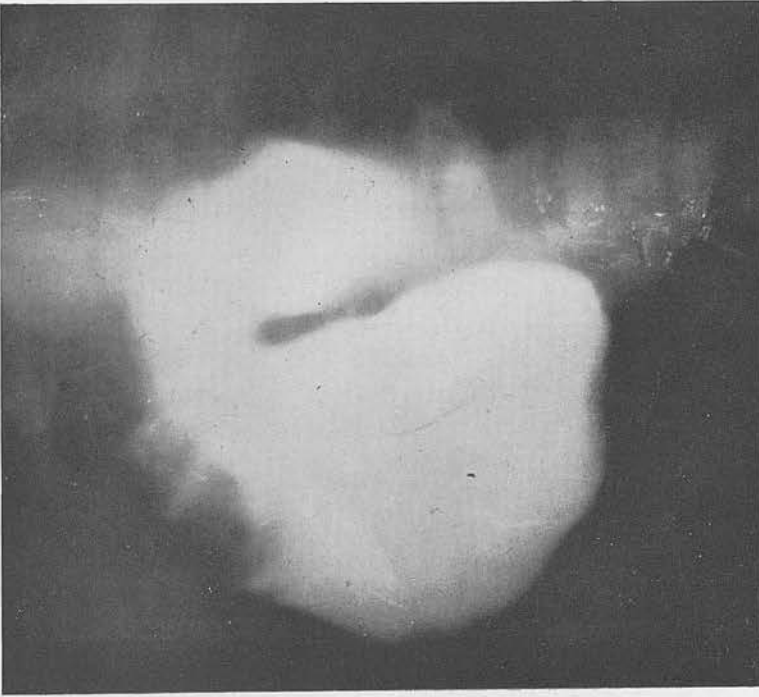


FIG. 1.—Dorso-ventral view of stomach in a suckling foal 5 minutes after barium meal.

[To face page 212]

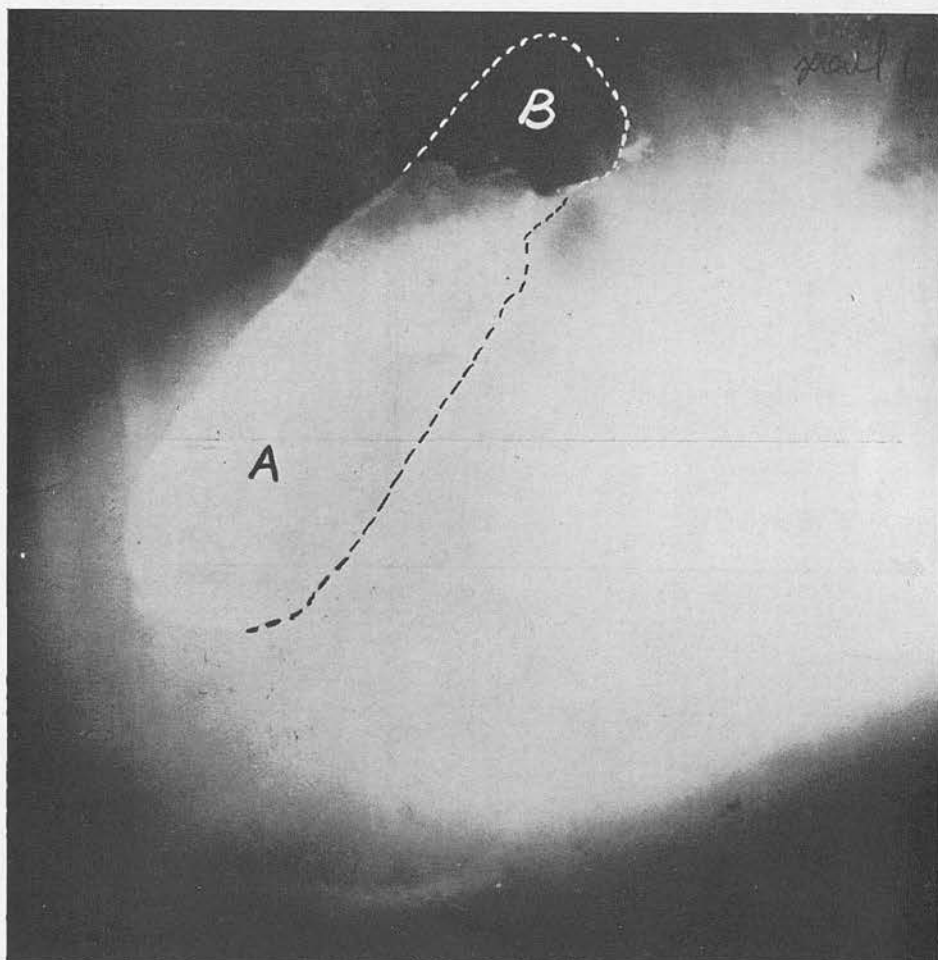


FIG. 1b.—Left lateral view of suckling foal. A, stomach; B, gas bubble.



FIG. 2.—Dorso-ventral view of stomach and duodenum in a suckling foal 30 minutes after barium meal.



FIG. 2a.—Dorso-ventral view of stomach (A) and duodenum (B) in a weaned foal 30 minutes after barium meal.





FIG. 3.—Dorso-ventral view of stomach and ileum of a suckling foal 4 hours after barium meal.

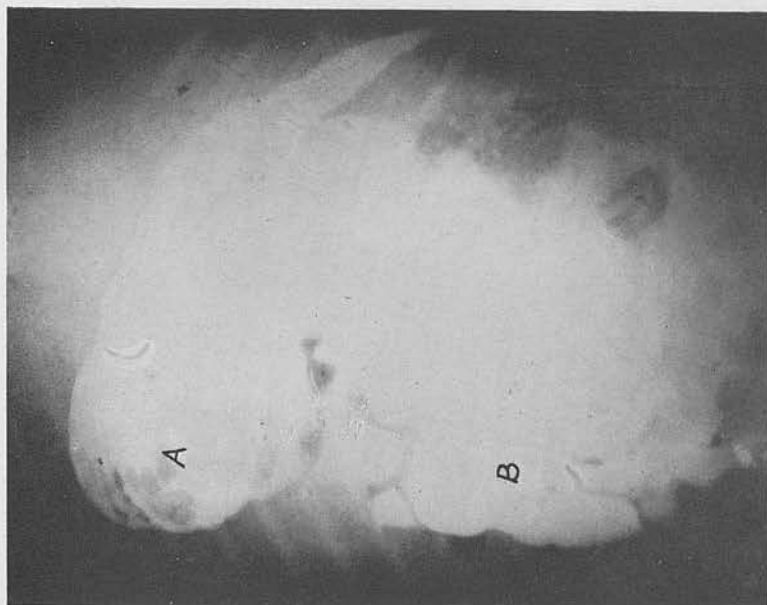


FIG. 3a.—Dorso-ventral view of stomach (A) and ileum (B) in a weaned foal 4 hours after barium meal.

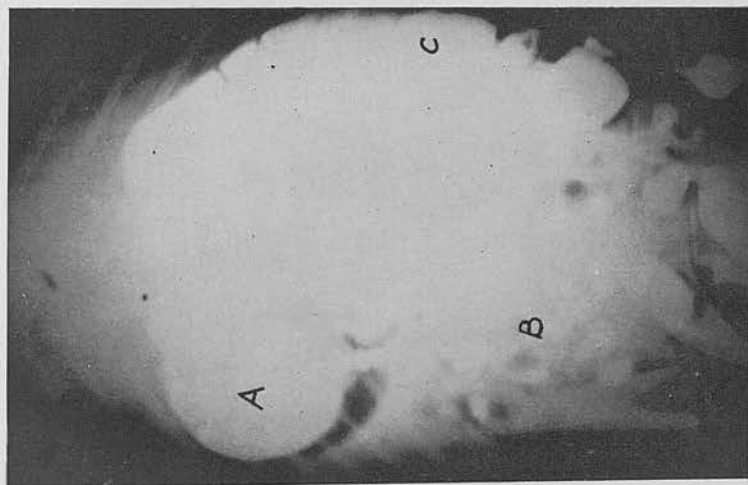


FIG. 4.—Dorso-ventral view of sucking foal 5½ hours after barium meal, showing stomach (A), ileum (B) and right ventral colon (C).

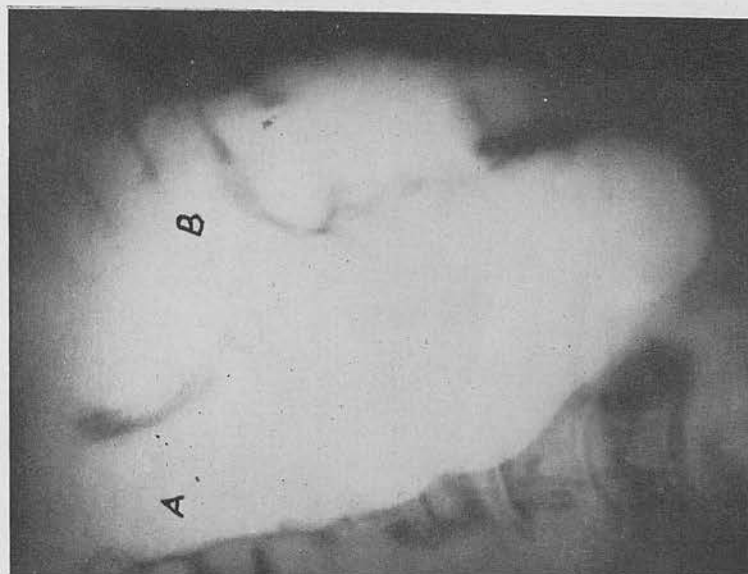


FIG. 4a.—Dorso-ventral view of weaned foal 7½ hours after barium meal, showing caecum (A) and right ventral colon (B).

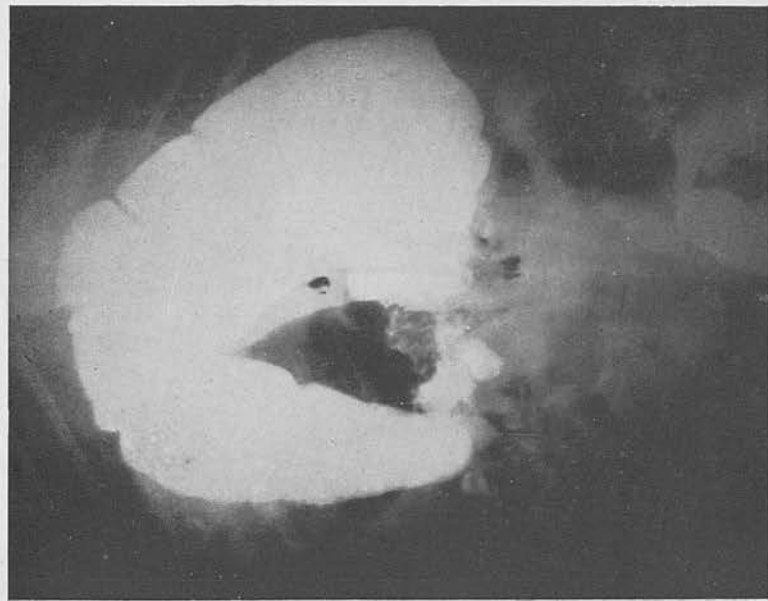


FIG. 5.—Dorso-ventral view of suckling foal 9 hours after barium meal, showing the large colon.

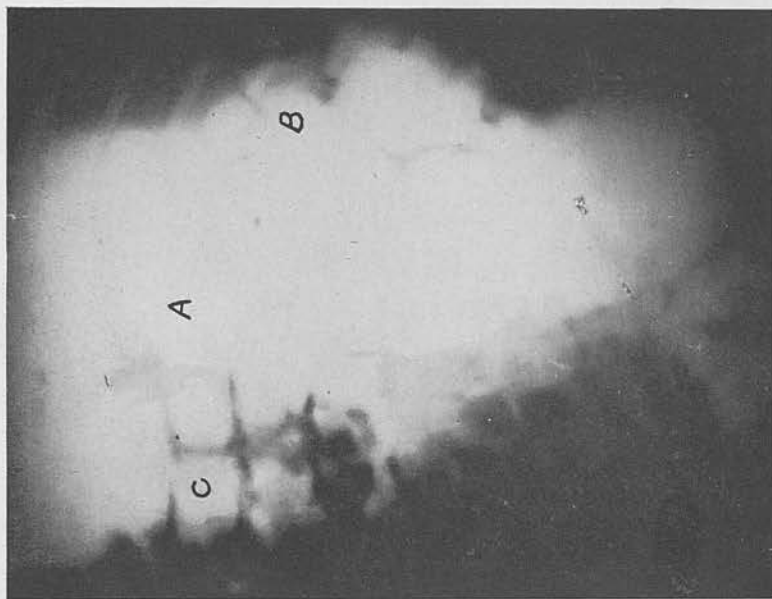


FIG. 5a.—Dorso-ventral view of a weaned foal 9 hours after barium meal, showing the caecal apex (A), the right ventral colon (B) and the left ventral colon (C).

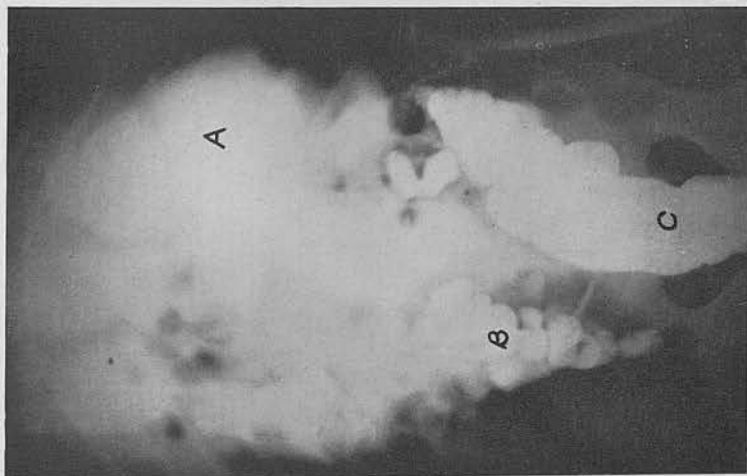
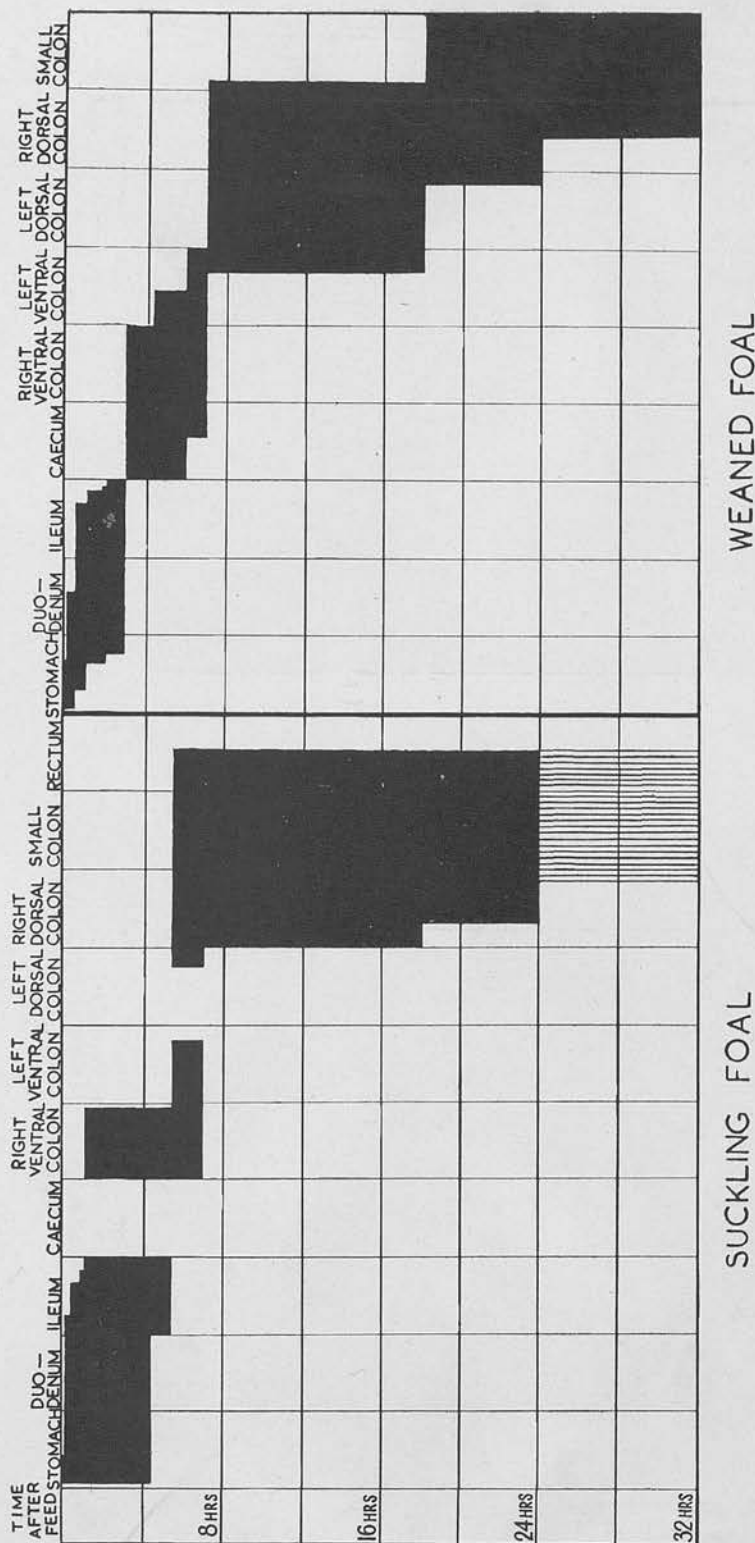


FIG. 6.—Dorso-ventral view of a suckling foal 24 hours after barium meal, showing the right dorsal colon (A), the small colon (B) and the rectum (C).



FIG. 6a.—Dorso-ventral view of a weaned foal 24 hours after barium meal, showing the right dorsal colon (A) and the small colon (B).



## PROGRESS OF A BARIUM MEAL THROUGH THE DIGESTIVE TRACT

FIG. 7.—Diagram to illustrate the rate of progress of a barium meal through the various parts of the digestive tract.

# CONTENTS

	PAGE
IBRAHIM, F. D., STELLA, G., and TALAAT, M. The Mechanism of Antidromic Vasodilatation . . . . .	189
DANIEL, P. M., PEABODY, C. N., and PRICHARD, MARJORIE M. L. Observations on the Circulation through the Cortex and the Medulla of the Kidney . . . . .	199
PICKFORD, M., and WATT, J. A. Comparison of some of the Actions of Adrenaline and Noradrenaline on the Kidney . . . . .	205
ALEXANDER, F., and BENZIE, D. A Radiological Study of the Digestive Tract of the Foal . . . . .	213
DELORME, E. J., MACPHERSON, A. I. S., MUKHERJEE, S. R., and ROWLANDS, S. Measurement of the Visceral Blood Volume in Dogs . . . . .	219

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FERMENTATIVE ACTIVITIES OF SOME MEMBERS OF  
THE NORMAL COCCAL FLORA OF THE HORSE'S LARGE  
INTESTINE

F. ALEXANDER, MARGARET J. D. MACPHERSON AND A. E. OXFORD  
*The Rowett Research Institute, Bucksburn, Aberdeenshire.*

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## FERMENTATIVE ACTIVITIES OF SOME MEMBERS OF THE NORMAL COCCAL FLORA OF THE HORSE'S LARGE INTESTINE

By

F. ALEXANDER, MARGARET J. D. MACPHERSON AND A. E. OXFORD

*The Rowett Research Institute, Bucksburn, Aberdeenshire.*

### INTRODUCTION

The high incidence of diseases of digestion in the horse has long been recognised. Dobberstein and Dinter (1941) found that of 1121 autopsies on horses they accounted for 49·4 per cent. In view of this fact it is surprising that fundamental information on the physiology of digestion in the horse is conspicuously lacking. One reason for this has been the surgical problems involved in preparing fistulæ into the various parts of the bowel. However, some of these problems have already been surmounted. Alexander and Donald (1949) described a technique for the fistulation of the cæcum and large colon, and more recently Alexander (1952) has found it possible to prepare a horse with fistulæ in the cæcum and dorsal and ventral colons. By means of such fistulated animals observations on the fermentative processes of the large bowel have been made, and some of the more active bacteria involved have been isolated.

Particular attention has been paid to cocci in view of the predominantly coccal microflora of other alimentary sites, such as the rumen of sheep and cattle (Hungate, 1950; Sijpesteijn, 1951), where active carbohydrate fermentation is proceeding. It was hoped also to throw some light on the anomalous behaviour of *Streptococcus equinus* (Andrewes and Horder, 1906) the characteristic streptococcus of horsedung, which ferments but few sugars when compared with other faecal streptococci (Orla-Jensen, 1943). By standard enrichment procedures several facultatively anaerobic streptococcal strains of wide fermentative powers have been isolated from colon sites in fistulated horses and also a Gram-negative coccus, which is very active in fermenting lactate, a normal fermentation product of faecal streptococci. This paper deals in detail with the fermentative behaviour of these bacteria and with the question of their identification.

### METHODS

*Experimental animals.* Three ponies were used, two (B and G) having fistulæ in the right dorsal and ventral colons (hereinafter referred to as colons 4 and 1 respectively) and the third (T) fistulated in colon 1 only. The animals were maintained on hay and oats throughout.

*Enrichment of bacteria in the colon contents.* Most attention was paid to the fermentation of lactose, and of cellobiose a possible

intermediate in cellulose degradation, which are not usually attacked by *Str. equinus*. Material from the colon was withdrawn in the morning, collected in warm and chemically clean beakers, taken immediately to the 39° incubator and strained through 4 layers of clean, sterile surgical gauze in order to remove the greater part of the plant debris. The filtrate was quickly distributed in 20 ml. lots, into sterile 6" test-tubes, each containing not more than 200 mg. of the solid, finely powdered sugar to be fermented previously sterilised by steam. After thorough mixing to dissolve the sugar, each test-tube was fitted with a clean and steam-sterilised cork bearing a bunsen-valve attachment, and all, save the unfermented controls which were immediately put in the cold room, were then incubated for 6 to 7 hours, when the fermentation was stopped by cooling to 0°. It was considered advisable not to continue the fermentations for a longer period in order to avoid an appreciable growth of chance contaminants.

*Analysis of fermentation liquors.* The pH as determined by the glass electrode was a sufficient indication of a good fermentation. It was invariably found that at values below pH 5.0 most of the sugar had disappeared. Lactic acid was determined colorimetrically (Hillig, 1942) and the total volatile fatty acid by repeated steam distillation after acidification. In some instances a viable bacterial count was made, both before and after fermentation in 1 per cent Bacto Yeast Extract agar medium containing 1 per cent lactose or cellobiose, by the roll-tube method. The roll-tubes were incubated for 2 days at 39° in a N<sub>2</sub>+5 per cent CO<sub>2</sub> atmosphere. Colonies which were almost invariably cocci were then picked off for microscopic examination and purification by replating in the usual way.

*Isolation of lactate-fermenting bacteria.* A highly-diluted colon liquor was judged to contain lactate-decomposing bacteria if marked disruption occurred in shake agar tubes after 24 hours incubation. The medium was composed of Na lactate, 2 per cent; Bacto Yeast Extract, 0.67 per cent; Bactopeptone, 0.33 per cent; K<sub>2</sub>HPO<sub>4</sub>, 0.33 per cent; KH<sub>2</sub>PO<sub>4</sub> 0.1 per cent; agar 2 per cent and sodium lactate as the source of carbohydrate. The initial pH was between 6 and 7. A control with no lactate in the medium never showed gas production even after several days' incubation.

For the isolation of the lactate fermenting bacteria, sterile Pasteur pipettes were inserted into the bottom of the disrupted shake cultures, where there was usually a free space containing some turbid liquid, and a few drops withdrawn for microscopic examination. If found to be rich in Gram-negative cocci, the rest of the liquid was transferred to a second shake culture from which blood agar plates incubated anaerobically at 40° were prepared. Purification was carried out by alternate culture of isolated colonies in liquid lactate medium, followed by replating on blood agar. When only Gram-negative diplococci were seen in stained

films and the final isolate also disrupted lactate agar, it was concluded that a pure culture had been obtained.

*Serological methods used for identification.* Precipitin tests were carried out with extracts made both by the acid method of Lancefield (1933) and by formamide extraction as described by Fuller (1938). The following group specific antisera were available: A, B, C, D, E, F, G, H, and K from Burroughs Wellcome & Co., and N from Dr. Mattick, Shinfield, Reading. Most useful information was obtained by the application of the Neufeld 'capsular-swelling' reaction used by Mirick, Thomas, Curnen and Horsfall (1944) for *Str. salivarius* and related strains.

Antisera homologous to the newly isolated horse streptococci were prepared by the method of Lancefield (1934). Cultures for injection into rabbits were prepared by re-suspending cells from 18 hour broth cultures in 0.85 per cent NaCl, containing 0.2 per cent formalin, to a volume equal to 5 per cent of that of the original culture. After 48 hours at 0° the bacterial suspensions were sterile. Immediately before use suspensions were diluted 20 times with saline. Doses of 1 ml. were injected intravenously into rabbits daily for 1 week, followed by a week's rest. Three courses of injections were given.

## RESULTS

### *Properties of Colon 1 and Colon 4 Liquors*

The respective micro-fauna of these liquors are quite distinct and serve to differentiate between them (Strelkow, 1939; Adam, 1951). There are also differences in chemical and physical properties. Strained colon 4 liquor is in general thicker and darker in colour than colon 1 liquor, and contains rather more material precipitable by dilute acetic acid (mucoprotein?). Both are essentially phosphate buffers (equivalent to about 0.15 per cent  $K_2HPO_4$ ) and are neutral in reaction. Colon 1 liquor also appears to contain a little bicarbonate, since its pH rises to 8.0-8.5 on standing. In each instance the buffering capacity is heightened by the presence of appreciable concentrations of volatile fatty acid anions.

### *Sugars other than lactose fermented by colon contents*

Pentoses were not readily attacked. Cellobiose, fructose, glucose, maltose, starch and sucrose were utilised by both colon 1 and colon 4 liquors, e.g. 0.25 per cent of dissolved substrate disappeared in 6 hours. Galactose and lactose were more readily attacked by colon 1 than by colon 4 liquor.

### *Lactose fermentations by colon 1 and colon 4 liquors*

In these experiments the colon 1 contents were from Pony T and the colon 4 contents from Pony B. The results of two separate 7 hour fermentation tests are summarised in Table I. Isolations of cultures were made from the colonies developing in the viable

counts underlined in Table I with results summarised in Table II. The seven streptococcal isolates denoted by asterisks in that table were kept in cultivation and a more extended study made of their fermentation reactions. Three of these strains were kept for the serological study recorded later.

TABLE I  
RESULTS OF FERMENTATION OF LACTOSE AND OTHER  
CARBOHYDRATES BY COLON LIQUORS 1 AND 4.

Experiment	Carbohydrate	Final pH		Viable bacterial count on lactose-yeast extract-agar (per ml; $\times 10^6$ )	
		Colon 1	Colon 4	Colon 1	Colon 4
I	None (after incubation)	7.0	No test	No test	1
	Lactose ... ..	4.85	5.05	68.75	420
	Cellobiose ... ..	4.7	4.6	150*	87.5*
	Mannitol ... ..	6.7	6.7	No test	No test
II	None (before incubation)	6.6	7.2	2	2.5
	None (after incubation) ...	7.0	6.8	No test	No test
	Lactose ... ..	4.7 ; 4.7	5.7 ; 5.8	90	15
	Cellobiose ... ..	4.6 ; 4.5	4.9 ; 4.8	No test	164.5*

\*On cellobiose-yeast-extract agar.

TABLE II  
NUMBER OF LACTOSE AND NON-LACTOSE FERMENTING  
STREPTOCOCCI FROM ISOLATED COLONIES.

Source of streptococci and kind of fermentation liquid examined	No. of colonies picked off	No. of lactose-fermenting strains isolated
Colon 1 ; lactose ... ..	5	5
Colon 4 ; lactose ... ..	9	7
Colon 1 ; before fermentation ... ..	7	6*
Colon 1 ; lactose ... ..	1	1*
Colon 4 ; before fermentation ... ..	2	0
Colon 4 ; lactose ... ..	3	0

\* Kept in subculture.

*Properties of the lactose-fermenting streptococci.* These streptococci were short-chained or diplococcal in appearance, the chains being embedded in a thin mucoid material, particularly on solid media. The strains were non-haemolytic and only one strain which quickly died out in sub-culture, fermented mannitol. The others fermented arabinose, glucose, galactose, maltose, lactose, trehalose,



raffinose, starch, dextrin and *æ*sculin with production of acid, chiefly *d*-lactic. After 5 days' incubation the pH which was initially 7.6 had fallen to between 4 and 5. The following were not fermented: xylose, rhamnose, glycerol, mannitol, dulcitol, sorbitol, inositol. One strain (J) fermented sucrose, one strain (A) fermented both inulin and salicin. The two strains (A, J) and a third (N) which gave typical fermentation reactions, were positive to the methyl red test, did not liquefy gelatin, hydrolysed Na hippurate and reduced methylene blue with clot in skim milk medium.

*Antigenic relationships of the lactose-fermenting streptococci.* Antigenic extracts prepared from strains A, J and N by the formamide method of Fuller (1938) gave positive precipitin reactions with homologous antiserum prepared against strain J. On the other hand they gave entirely negative results when tested against all commercial sera, or against antisera from sheep's rumen streptococci (MacPherson, 1952). These results were most convincingly confirmed by means of the Neufeld capsular swelling reaction. Only in the presence of the homologous antiserum was the capsule clearly outlined as a greyish zone surrounding each pair or short chain of cocci. The addition of antiserum J to the colon liquor itself revealed definite capsulation in a small proportion of the cocci present. With normal serum no such capsulation could be demonstrated (Figs. 1 and 2).

FIG. 1

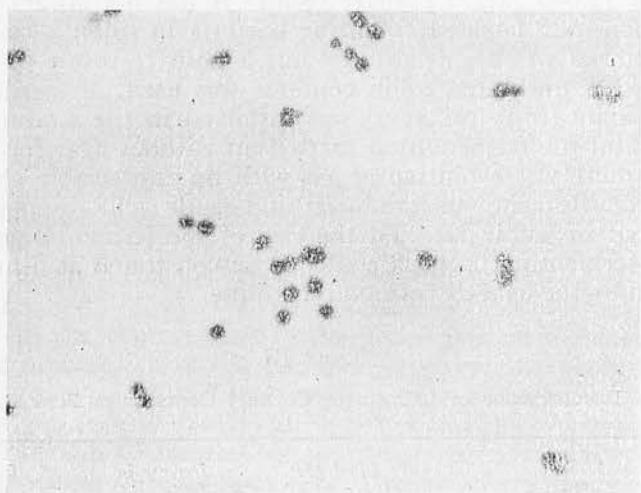


FIG. 1. Horse colon streptococcus strain J and normal rabbit serum. No obvious capsule.  $\times 2500$

#### *Lactate fermentations by colon 1 and colon 4 liquors*

On all occasions with all 3 ponies disruption of the agar occurred when the colon 1 contents were diluted 600 times with



FIG. 2



FIG. 2. *Streptococcus J* and homologous antiserum, showing clear delineation of the capsule.  $\times 2500$

Both were wet preparations stained with methylene blue.

sterile water before testing. On some occasions dilutions of 10,000 or even 25,000 gave a positive result. It was not always possible to demonstrate lactate-fermenting bacteria in colon 4 contents if the material was highly diluted but a positive result was always obtained if undiluted colon contents was used. If sterile Na or  $\text{NH}_4$  lactate (0.35 per cent) was dissolved in the strained colon liquor and the fermentation carried on without agar for 7 hours at  $39^\circ$  there was evolution of gas with no appreciable change in pH. Volatile acid was produced and lactic acid disappeared as indicated in Table III. At the end of the fermentation period lactate-fermenting bacteria could be demonstrated at dilutions of 100,000 by the agar-disruption technique.

TABLE III

DECOMPOSITION OF LACTATE BY COLON 1 CONTENTS OF PONY B.

Substrate					Final pH after 7 hours' fermentation	Increase in volatile acid, calc. as acetic mg%	Loss of lactic acid mg%
None	...	...	...	...	6.1	36	None
Na lactate	...	...	...	...	6.8	216	150
$\text{NH}_4$ lactate	...	...	...	...	6.2	198	220
Free lactic acid	...	...	...	...	6.2	114	150

*Properties of the lactate-fermenting Gram-negative cocci.* These organisms were strict anaerobes with rather exacting growth requirements, blood agar or cooked meat medium being necessary for their continued propagation. No carbohydrates were fermented and among all the hydroxy and dicarboxylic acids tried as substrates, gas was produced only from lactate, and to a lesser extent from tartrate. The colonial appearance on agar and most other properties were exactly as described in detail by Johns (1951) for the organism obtained from the sheep's rumen, and presumed to be *Veillonella gazogenes*. In view of this and of the frequent occurrence of this coccus in intestinal sites no further investigation of our equine strain was considered necessary.

#### DISCUSSION

It is clear that the *Str. equinus* of faecal origin, which ferments only a few carbohydrates, is by no means the only streptococcus to be found in the colon of the horse. The streptococci which we have isolated, in so far as fermentation reactions are concerned, resemble the well-known *Str. bovis* which has wide fermentative powers (Orla-Jensen, 1943), but on serological grounds seem to fall into a group of their own. They are capsulated and their presence, either in pure or in mixed cultures or in the colon fluids, can conveniently be demonstrated by means of the Neufeld capsular swelling reaction provided homologous antiserum is used. We are of the opinion that this very simple serological test might be more widely used in the identification of encapsulated streptococci in the natural situations in which they occur. A further study (MacPherson, 1952) along these lines is in progress with other animals.

The chief fermentation product of these colon streptococci is lactic acid which is not usually found in colon liquor. Consequently, the presence in the colon of the ubiquitous *Veillonella gazogenes*, which ferments no sugars but only lactate to volatile fatty acids is not unexpected. Since a more vigorous fermentative activity is observed in the ventral than in the dorsal part of the colon, the greater concentration of the Gram-negative coccus in the former site and the presence of bicarbonate ions in quantity in colon 1 liquor only is readily explained. It seems unlikely, however, that *V. gazogenes* can be responsible for the production of the greater part of the high volatile fatty acid content which has been estimated at 0.3 to 0.7 per cent of colon liquor (Alexander, 1952), partly because it seems to be present in insufficient numbers and partly because the colon does not normally contain large volumes of gas. The fermentation of lactate by *V. gazogenes* would undoubtedly produce hydrogen as well as CO<sub>2</sub> (Johns, 1951). It may be that lactate is very rapidly absorbed from the colon and only small amounts remain for fermentation with *V. gazogenes*. The fermentative and absorptive processes of the horse's colon evidently

need a much fuller study, which one of us (F.A.) hopes to carry out in the near future.

A point of some interest is the fact that the common streptococcus of horse dung differs so markedly from the organisms isolated from the colon. It is possible that selective feeding on the part of ciliate protozoa, which are present in large numbers in colon 4 (Adam, 1951), may account for this anomaly.

#### SUMMARY

Streptococci capable of fermenting lactose and starch have been isolated from the large colon contents of fistulated ponies.

They form a capsule which is easily demonstrated by the capsular swelling reaction described by Neufeld.

They can be differentiated serologically from other types of streptococci.

The colon also contains the Gram-negative coccus *Veillonella gazogenes*, an anaerobic fermenter of lactate.

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## Glucuronide Decomposition in the Digestive Tract

THE discovery of an enzyme decomposing glucuronide in the rumen of the sheep<sup>1</sup> has been followed by the detection of similar enzymes in the large intestine of the horse<sup>2</sup> and in locust crop liquor<sup>3</sup>. It seemed possible that such enzymes are concerned in the digestion of polysaccharides containing  $\beta$ -linked glucuronic acid residues, such as hemicellulose and hyaluronic acid, and a survey was therefore made of their occurrence in different parts of the alimentary tract in various species of animal. Typical results are shown in the accompanying table. It should be pointed out that these results are only of qualitative value, since the enzyme assays were carried out under conditions which are optimal for the sheep rumen enzyme<sup>1</sup>, but are not necessarily so for the enzymes from other sources. Moreover, in the rumen, omasum, caecum and colon, the enzymes may well be of microbial origin, as has been shown for the sheep rumen enzyme<sup>1</sup>, and therefore surrounded by membranes of varying toughness and permeability. A further factor which was not controlled was the diet of the animals, some of the material being obtained from the slaughter-house.

In spite of the reservations made above, the results in the table are of considerable interest in showing the occurrence of very active enzymes of this type in the digestive tracts of all species of animal studied. So far as any generalizations are possible, it may be said that enzyme activity was appreciable in the fore-stomachs of ruminants, and very high in the large bowel of all species. Activity was low or lacking in the stomach, abomasum or gizzard. Appreciable activity was frequently noted in the small intestine. This may have been due to secretion of animal tissue  $\beta$ -glucuronidase into the lumen or to sloughing of epithelial cells.

Enzymes from many sources were tested for inhibition by 0.00025 *M* saccharo-1:4-lactone. In this concentration, the lactone causes complete inhibition of mouse liver  $\beta$ -glucuronidase, but only 50 per cent inhibition of the enzyme from the rumen of the sheep<sup>4</sup>. In the present experiments it caused

DISTRIBUTION OF ENZYMES DECOMPOSING GLUCURONIDE IN THE ALIMENTARY TRACT  
 $\mu$ gm. Phenolphthalein liberated in 1 hr. at 37° and pH 6.1 (phosphate buffer) from 0.0005 M phenolphthalein glucuronide by 1 ml. strained  
 gut content)

Animal	Rumen	Omasum	Stomach or abomasum	Duodenum	Ileum	Cæcum	Colon (proximal)	Colon (distal)
Sheep	112	161	0	174	143	3,880	2,700	3,425
Cow	31	166	37	82	65	1,168	154	1,292
Horse	—	—	0	not done	2	107	515	229
Rabbit	—	—	0	19	9	3,000	2,100	—
Rat	—	—	0	114	127	4,300	2,800	189
Pig	—	—	20	0	45	968	1,020	—
Cat	—	—	0	126	116	392	331	—
	Crop	Gizzard						
Fowl	46	0	empty	221	363	8,080	—	189

complete inhibition of the enzyme in the duodenum and 35-64 per cent inhibition of the enzyme in the rumen or large intestine.

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